

10/500796
10/500796 06 JUL 2004

REC'D 14 JUN 2002

WIPO PCT

Pl 819731

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

June 12, 2002

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 09/826,115

FILING DATE: April 04, 2001

RELATED PCT APPLICATION NUMBER: PCT/US02/10764



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

T. Wallace
T. WALLACE
Certifying Officer

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

BEST AVAILABLE COPY

04/04/01

4-501

EXPRESS MAIL NO.: EL 491887575 US
 ATTORNEY DOCKET NO. 14114.0332U3
 SERIAL NO. Unassigned
 PAGE 1 OF 3 (04/04/01)

CONTINUING APPLICATION TRANSMITTAL FORM
 (37 C.F.R. § 1.53(b))

ANTICIPATED CLASSIFICATION OF THIS APPLICATION:

PRIOR APPLICATION: 09/701,536

CLASS:

SUBCLASS:

EXAMINER: Unassigned

ART UNIT: Unassigned

To the Assistant Commissioner for Patents:

This is a request for filing a continuation-in-part application under 37 C.F.R. § 1.53(b), of pending prior application Serial No. 09/701,536, filed on November 29, 2000, which is a national phase application filed from international patent application PCT/US99/12298, filed June 3, 1999, which claims priority to Provisional application Serial No. 60/087,908, filed June 4, 1998 of Gwong-Jen J. Chang for "NUCLEIC ACID VACCINES FOR PREVENTION OF FLAVIVIRUS INFECTION."

If any extension of time is necessary for the filing of this application, including any extension of time necessary in the prior application for maintaining copendency between the prior application and this application, and such extension has not otherwise been requested, Applicant hereby petitions for such an extension in the prior application.

1. Enclosed is a specification as prescribed by 35 U.S.C. § 112 containing a description pursuant to § 1.71 and at least one claim pursuant to § 1.75, and any drawing required by § 1.81(a).

2. The inventor of the invention being claimed in this application is: Gwong-Jen J. Chang

3A. ☐ This application is a continuation or divisional application (not a continuation-in-part) that:

- (i) names as inventors the same or fewer than all of the inventors named in the prior application; and
- (ii) contains no matter that would have been new matter in the prior application; and
- (iii) a copy of the executed oath or declaration filed in the prior application, showing the signature or an indication thereon that it was signed:

☐ is enclosed.
☐ will follow.

☐ This application is being filed by less than all the inventors named in the prior application. In accordance with 37 C.F.R. § 1.53(d) (4), the Commissioner is requested to delete the name(s) of the following persons who are not inventors of the invention being claimed in this application:

--OR--

3B. ☒ This application is a continuation-in-part application, or a continuation or divisional application naming an inventor not named in the prior application. A newly executed oath or declaration:

☒ is enclosed.
☐ will follow.

4. ☐ Amend the Title of the Invention as follows:

5. ☐ Amend the specification by inserting before the first line the sentence:

6. ☐ Priority of foreign application number is claimed under 35 U.S.C. § 119.

☐ The certified copy has been filed in prior application Serial No. , filed on .

USPTO
 09/826115
 04/04/01

0925115-040404

7. ☐ Cancel in this application original claims of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

8. ☐ A preliminary amendment is enclosed.

9. ☒ New formal drawings are enclosed.

10. ☒ Also enclosed is/are: Sequence listing (46 pages); Sequence listing diskette; and form entitled Authorization to Treat Reply Requiring Extension of Time as Incorporating Petition for Extension of Time and Payment of Extension of Time Fee.

11. ☐ The prior application is assigned of record to:

12. ☐ Applicant claims small entity status. See 37 C.F.R. § 1.27.

13. The filing fee is calculated as follows:

TOTAL CLAIMS	43 - 20 =	23	x \$18.00	\$414.00
INDEPENDENT CLAIMS	1 - 3 =	0	x \$80.00	\$0.00
MULTIPLE DEPENDENT CLAIM(S) (IF APPLICABLE) =			+ \$270.00	\$0.00
BASIC FEE =				\$710.00
TOTAL OF ABOVE CALCULATIONS =				\$1,124.00
REDUCTION BY ½ FOR FILING BY SMALL ENTITY (NOTE 37 C.F.R. § 1.9, § 1.27, § 1.28)				\$
TOTAL NATIONAL FEE =				\$1,124.00

14. ☒ A check in the amount of \$1,124.00 is enclosed.

15. ☒ The Commissioner is hereby authorized to charge any fees which may be required under 37 C.F.R. § 1.16 and § 1.17, or credit any overpayment to Deposit Account No. 14-0629.

16. ☒ The Power of Attorney in the prior application is to: Needle & Rosenberg, P.C.

- a. ☒ The Power of Attorney appears in the original papers in the prior application.
- b. ☐ Since the Power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. ☒ Address all future correspondence to: (May only be completed by applicant, or attorney or agent of record.)

Mary L. Miller, Esq.
NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, GA 30303-1811

17. I hereby verify that all statements made herein of my own knowledge are true; and further that these statements were made with the knowledge that willful false statements and the like are made punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Mary L. Miller
REG. NO. 39,303

Mary L. Miller
SIGNATURE

April 4, 2001
DATE

- ☐ Inventor(s)
☐ Assignee of complete interest
☒ Attorney or agent of record
☐ Filed under 37 C.F.R. § 1.34(a)
(Registration No. is acting under 37 C.F.R. § 1.34(a):

EXPRESS MAIL NO. EL 491887575 US

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Invoice No. EL 491887575 US in an envelope addressed to: BOX PATENT APPLICATION, Assistant Commissioner for Patents, Washington, D.C. 20231, on the date shown below.

Everardo McFarlane
Everardo McFarlane

4-4-01
DATE

EXPRESS MAIL NO. EL491587108US
ATTORNEY DOCKET NO. 14114.0332U3
PATENT

09826115-040401
F040401-51192860

TO ALL WHOM IT MAY CONCERN:

5 Be it known that I, Gwong-Jen J. Chang, a citizen of the United States of
America, residing at 4237 Beaver Creek Drive, Ft. Collins, CO 80526, U.S.A., have
invented new and useful improvements in

**NUCLEIC ACID VACCINES FOR PREVENTION
OF FLAVIVIRUS INFECTION**

10 for which the following is a specification.

**NUCLEIC ACID VACCINES FOR PREVENTION
OF FLAVIVIRUS INFECTION**

5 This application is a continuation-in-part of, and claims the benefit of, U.S.
application Serial No. 09/701,536, filed November 29, 2000, which status is pending and
which is a national stage application of international application Serial No.
PCT/US99/12298, filed June 3, 1999 from U.S. provisional application Serial No.
60/087908, filed June 4, 1998, which applications are hereby incorporated herein in their
entirety by reference.

10

Field of the Invention

15 This invention relates to novel vaccines, diagnostics and methods of using both
in the treatment and prevention of the diseases caused by flaviviruses. In particular,
the vaccines are recombinant nucleic acids which contain genes for structural proteins
of flaviviruses, such as Japanese encephalitis virus (JEV), West Nile virus (WNV) or
related flaviviruses. These vaccines serve as a transcriptional unit for the biosynthesis
of the virus protein antigens when administered *in vivo*. The diagnostics are
compositions containing antigens produced from the recombinant nucleic acids that can
be used to detect flavivirus infection.

20

Background of the Invention

Flaviviruses are members of the genus *Flavivirus*, which is classified within the
family Flaviviridae. The flaviviruses are largely pathogenic to humans and other
mammals. Flaviviruses that inflict disease upon humans and animals include Alfuy,
Apoi, Aroa, Bagaza, Banzi, Batu Cave, Bouboui, Bukalasa bat, Bussuquara,
25 Cacipacore, Carey Island, Cowbone Ridge, Dakar bat, Dengue (serotypes 1, 2, 3 and 4),
Edge Hill, Entebbe bat, Gadgets Gully, Iguape, Ilheus, Israel turkey

- meningoencephalitis, Japanese encephalitis, Jugra, Jutiapa, Kadam, Karshi, Kedougou, Kokobera, Koutango, Kunjin, Kyasanur Forest disease, Langat, Meaban, Modoc, Montana myotis leukoencephalitis, Murray Valley encephalitis, Naranjal, Negishi, Ntaya, Omsk hemorrhagic fever, Phnom Penh bat, Potiskum, Powassan, Rio Bravo,
- 5 Rocio, Royal Farm, Russian spring summer encephalitis, Saboya, Sal Vieja, San Perlita, Saumarez Reef, Sepik, Sokuluk, Spondweni, St. Louis encephalitis, Stratford, Tick-borne encephalitis - central European subtype, Tick-borne encephalitis - far eastern subtype, Tembusu, THCAr, Tyulenyi, Uganda S, Usutu, West Nile, Yaounde, Yellow fever, Yokose, Ziki, Cell fusing agent and other related flaviviruses, as listed in
- 10 Kuno et al. (*J. Virol.* 72: 73-83 (1998)).

- The flaviviruses contain the following three structural proteins: prM/M, the premembrane and membrane protein; E, the envelope protein; and C, the capsid protein. (Monath, in *Virology* (Fields, ed.), Raven Press, New York, 1990, pp. 763-814; Heinz and Roehrig, in *Immunochemistry of Viruses II: The Basis for*
- 15 *Serodiagnosis and Vaccines* (van Regenmortel and Neurath, eds.), Elsevier, Amsterdam, 1990, pp. 289-305). M has a molecular weight (MW) of about 7-8 kilodaltons (kDa) and E has a MW of about 55-60 kDa. M is synthesized as a larger precursor termed prM. The pr portion of prM is removed when prM is processed to form M protein in mature virions. M and E are located in the membrane of the
- 20 flavivirus particle, and so have long been considered to constitute important immunogenic components of the viruses.

- The flaviviruses are RNA viruses comprising single stranded RNA having a length, among the various species, of about 10 kilobases (kb). The C protein, with a MW of 12-14 kDa, complexes with the RNA to form a nucleocapsid complex. Several
- 25 nonstructural proteins are also encoded by the RNA genome which are termed NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The genome is translated within the host cell as a polyprotein, then processed co- or post-translationally into the individual gene products by viral- or host-specific proteases (Figure 1).

The nucleotide sequences of the genomes of several flaviviruses are known, as summarized in U. S. Patent No. 5,494,671. That for JEV is provided by Sumiyoshi et al. (*Virology* 161: 497-510 (1987)) and Hashimoto et al. (*Virus Genes* 1: 305-317 (1988)). The nucleotide sequences of the virulent strain SA-14 of JEV and the
5 attenuated strain SA-14-14-2, used as a vaccine in the People's Republic of China, are compared in the work of Nitayaphan et al. (*Virology* 177: 541-552 (1990)).

Nucleotide sequences encoding the structural proteins of other flavivirus species are also known. In many cases, the sequences for the complete genomes have been reported. The sequences available include dengue serotype 1 virus, dengue serotype 2
10 virus (Deubel et al., *Virology* 155: 365-377 (1986); Gruenberg et al., *J. Gen. Virol.* 69: 1391-1398 (1988); Hahn et al. *Virology* 162: 167-180 (1988)), dengue serotype 3 virus (Osatomi et al., *Virus Genes* 2: 99-108 (1988)), dengue serotype 4 virus (Mackow et al., *Virology* 159: 217-228 (1987), Zhao et al., *Virology* 155: 77-88 (1986)), West Nile virus (Lanciotti et al., *Science* 286: 2331-2333 (1999)), Powassan virus (Mandl et al.,
15 *Virology* 194: 173-184 (1993)) and yellow fever virus (YFV) (Rice et al., *Science* 229: 726-733 (1985)).

Many flaviviruses, including St. Louis encephalitis virus (SLEV), WNV and JEV, are transmitted to humans and other host animals by mosquitoes. They therefore occur over widespread areas and their transmission is not easily interrupted or
20 prevented.

West Nile fever is a mosquito-borne flaviviral infection that is transmitted to vertebrates primarily by various species of *Culex* mosquitoes. Like other members of the Japanese encephalitis (JE) antigenic complex of flaviviruses, including JE, SLE and Murray Valley encephalitis (MVE) viruses, WNV is maintained in a natural cycle
25 between arthropod vectors and birds. The virus was first isolated from a febrile human in the West Nile district of Uganda in 1937 (Smithburn et al., *Am. J. Trop. Med. Hyg.* 20: 471-492 (1940)). It was soon recognized as one of the most widely distributed

0922615-04044

flaviviruses, with its geographic range including Africa, the Middle East, Western Asia, Europe and Australia (Hubalek et al., *Emerg. Infect. Dis.* 5: 643-50 (1999)). Clinically, West Nile fever in humans is a self-limited acute febrile illness accompanied by headache, myalgia, polyarthropathy, rash and lymphadenopathy (Monath and Tsai, in *Clinical Virology*, (Richman, Whitley and Hayden eds.), Churchill-Livingstone, New York, 1997, pp. 1133-1186). Acute hepatitis or pancreatitis has been reported on occasion and cases of WNV infection in elderly patients are sometimes complicated by encephalitis or meningitis (Asnis et al., *Clin. Infect. Dis.* 30: 413-418 (2000)). Thus, infection by WNV is a serious health concern in many regions of the world.

- 10 The geographical spread of the disease, particularly the introduction of WNV into the U.S. in 1999, has greatly increased awareness of the human and animal health concerns of this disease. Between late August and early September 1999, New York City and surrounding areas experienced an outbreak of viral encephalitis, with 62 confirmed cases, resulting in seven deaths. Concurrent with this outbreak, local health
- 15 officials observed increased mortality among birds (especially crows) and horses. The outbreak was subsequently shown to be caused by WNV, based on monoclonal antibody (Mab) mapping and detection of genomic sequences in human, avian and mosquito specimens (Anderson et al., *Science* 286: 2331-2333 (1999); Jia et al., *Lancet* 354: 1971-1972 (1999); Lanciotti et al., *Science* 286: 2333-2337 (1999)). Virus activity
- 20 detected during the ensuing winter months indicated that the virus had established itself in North America (*Morb. Mortal. Wkly. Rep.* 49: 178-179 (2000); Asnis et al., *Clin. Infect. Dis.* 30: 413-418 (2000); Garmendia et al., *J. Clin. Micro.* 38: 3110-3111 (2000)). Surveillance data reported from the northeastern and mid-Atlantic states during the year 2000 confirmed an intensified epizootic/epidemic transmission and a
- 25 geographic expansion of the virus with documentation of numerous cases of infection in birds, mosquitoes and horses, as well as cases in humans (*Morb. Mortal. Wkly. Rep.* 49: 820-822 (2000)).

Japanese encephalitis virus (JEV) infects adults and children and there is a high mortality rate among infants, children and the elderly in areas of tropical and subtropical Asia (Tsai et al., in Vaccines (Plotkin, ed.) W.B. Saunders, Philadelphia, Pa, 1999, pp. 672-710). Among survivors, there are serious neurological consequences, related to the symptoms of encephalitis, that persist after infection. In more developed countries of this region, such as Japan, the Republic of China (Taiwan) and Korea, JEV has been largely controlled by use of a vaccine of inactivated JEV. Nevertheless, it is still prevalent in other countries of the region.

Dengue fever and dengue hemorrhagic fever (DF/DHF) are caused by dengue virus, which is also a mosquito-borne flavivirus. There are four antigenically related, but distinct, dengue virus serotypes, (DEN-1, DEN-2, DEN-3 and DEN-4), all of which can cause DF/DHF. Symptoms of DF, the mild form of dengue-related disease, include

fever, rash, severe headache and joint pain. Mortality among those subjects suffering from DF is low; however, among those subjects suffering from DHF, mortality can be as high as 5%. From available evidence, more than 3 million cases of DHF and 58,000 deaths have been attributed to DHF over the past 40 years, making DHF a major emerging disease (Halstead, in *Dengue and Dengue Hemorrhagic Fever* (Gubler and Kuno, eds.) CAB International, New York, NY, (1997) pp 23-44). Nevertheless, despite decades of effort, safe and effective vaccines to protect against dengue virus infection are not yet available.

Yellow fever is prevalent in tropical regions of South America and sub-Saharan Africa and is transmitted by mosquitos. Infection leads to fever, chills, severe headache and other pains, anorexia, nausea and vomiting, with the emergence of jaundice. A live virus vaccine, 17D, grown in infected chicken embryos, is considered safe and effective. Nevertheless, there remains a need for a vaccine that is stable under adverse conditions, such as are commonly encountered in the tropical regions of Africa and the Americas where the vaccine is most needed.

A recombinant flavivirus which is a chimera between two flaviviruses is disclosed in PCT publication WO 93/06214. The chimera is a construct fusing non-structural proteins from one "type," or serotype, of dengue virus or a flavivirus, with structural proteins from a different "type," or serotype, of dengue virus or other flavivirus.

Several recombinant subunit and viral vaccines have been devised in recent years. U. S. Patent No. 4,810,492 describes the production of the E glycoprotein of JEV for use as the antigen in a vaccine. The corresponding DNA is cloned into an expression system in order to express the antigen protein in a suitable host cell such as *E. coli*, yeast, or a higher organism cell culture. U. S. Patent No. 5,229,293 discloses recombinant baculovirus harboring the gene for JEV E protein. The virus is used to

infect insect cells in culture such that the E protein is produced and recovered for use as a vaccine.

- U. S. Patent No. 5,021,347 discloses a recombinant vaccinia virus genome into which the gene for JEV E protein has been incorporated. The live recombinant
- 5 vaccinia virus is used as the vaccine to immunize against JEV. Recombinant vaccinia viruses and baculoviruses in which the viruses incorporate a gene for a C-terminal truncation of the E protein of dengue serotype 2, dengue serotype 4 and JEV are disclosed in U.S. Patent 5,494,671. U. S. Patent 5,514,375 discloses various recombinant vaccinia viruses which express portions of the JEV open reading frame
- 10 extending from prM to NS2B. These pox viruses induced formation of extracellular particles that contain the processed M protein and the E protein. Two recombinant viruses encoding these JEV proteins produced high titers of neutralizing and hemagglutinin-inhibiting antibodies, and protective immunity, in mice. The extent of these effects was greater after two immunization treatments than after only one.
- 15 Recombinant vaccinia virus containing genes for the prM/M and E proteins of JEV conferred protective immunity when administered to mice (Konishi et al., *Virology* 180: 401-410 (1991)). HeLa cells infected with recombinant vaccinia virus bearing genes for prM and E from JEV were shown to produce subviral particles (Konishi et al., *Virology* 188: 714-720 (1992)). Dmitriev et al. reported immunization of mice with a
- 20 recombinant vaccinia virus encoding structural and certain nonstructural proteins from tick-borne encephalitis virus (*J. Biotechnology* 44: 97-103 (1996)).

- Recombinant virus vectors have also been prepared to serve as virus vaccines for dengue fever. Zhao et al. (*J. Virol.* 61: 4019-4022 (1987)) prepared recombinant vaccinia virus bearing structural proteins and NS1 from dengue serotype 4 and achieved
- 25 expression after infecting mammalian cells with the recombinant virus. Similar expression was obtained using recombinant baculovirus to infect target insect cells (Zhang et al., *J. Virol.* 62: 3027-3031(1988)). Bray et al. (*J. Virol.* 63: 2853-2856 (1989)) also reported a recombinant vaccinia dengue vaccine based on the E protein

gene that confers protective immunity to mice against dengue encephalitis when challenged. Falgout et al. (*J. Virol* 63: 1852-1860 (1989)) and Falgout et al. (*J. Virol.* 64: 4356-4363 (1990)) reported similar results. Zhang et al. (*J. Virol* 62: 3027-3031 (1988)) showed that recombinant baculovirus encoding dengue E and NS1 proteins
5 likewise protected mice against dengue encephalitis when challenged. Other combinations in which structural and nonstructural genes were incorporated into recombinant virus vaccines failed to produce significant immunity (Bray et al., *J. Virol.* 63: 2853-2856 (1989)). Also, monkeys failed to develop fully protective immunity to dengue virus challenge when immunized with recombinant baculovirus expressing the
10 E protein (Lai et al. (1990) pp. 119-124 in F. Brown, R. M. Chancok, H. S. Ginsberg and R. Lerner (eds.) Vaccines 90: Modern approaches to new vaccines including prevention of AIDS, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Immunization using recombinant DNA preparations has been reported for SLEV and dengue-2 virus, using weanling mice as the model (Phillpotts et al., *Arch.*
15 *Virol.* 141: 743-749 (1996); Kochel et al., *Vaccine* 15: 547-552 (1997)). Plasmid DNA encoding the prM and E genes of SLEV provided partial protection against SLEV challenge with a single or double dose of DNA immunization. In these experiments, control mice exhibited about 25% survival and no protective antibody was detected in the DNA-immunized mice (Phillpotts et al., *Arch. Virol.* 141: 743-749 (1996)). In mice
20 that received three intradermal injections of recombinant dengue-2 plasmid DNA containing prM, 100% developed anti-dengue-2 neutralizing antibodies and 92% of those receiving the corresponding E gene likewise developed neutralizing antibodies (Kochel et al., *Vaccine* 15: 547-552 (1997)). Challenge experiments using a two-dose schedule, however, failed to protect mice against lethal dengue-2 virus challenge.

25 The vaccines developed to date for immunizing against infection by JEV, SLEV, dengue virus and other flaviviruses have a number of disadvantages and problems attending their use. Inactivated vaccine is costly and inconvenient to prepare. In addition, any such vaccine entails the risk of allergic reaction originating from

proteins of the host cell used in preparing the virus. Furthermore, such vaccines present considerable risk to the workers employed in their production. Candidate attenuated JEV vaccines are undergoing clinical trials, but as of 1996 have not found wide acceptance outside of the People's Republic of China (Hennessy et al., *Lancet* 347: 1583-1586 (1996)).

Recombinant vaccines based on the use of only certain proteins of flaviviruses, such as JEV, produced by biosynthetic expression in cell culture with subsequent purification or treatment of antigens, do not induce high antibody titers. Also, like the whole virus preparations, these vaccines carry the risk of adverse allergic reaction to antigens from the host or to the vector. Vaccine development against dengue virus and WNV is less advanced and such virus-based or recombinant protein-based vaccines face problems similar to those alluded to above.

There is therefore a need for vaccines or improved vaccines directed against flaviviruses such as yellow fever virus, dengue virus, JEV, SLEV and WNV which are inexpensive to prepare, present little risk to workers involved in their manufacture, carry minimal risk of adverse immunological reactions due to impurities or adventitious immunogenic components and are highly effective in eliciting neutralizing antibodies and protective immunity. There is furthermore a need for a vaccine against JEV, WNV and related flaviviruses that minimizes the number of immunizing doses required.

Many of the shortcomings of the current art as described in detail for the production of vaccines also apply to the production of antigens and antibodies to be used for the production of immunodiagnosics. Particularly, the concurrent risks and costs involved in the production of antigens from viruses and the failure of most currently available recombinantly expressed antigens to elicit effective immune responses are paralleled in the field of immunodiagnosics by the same risks, high costs and a corresponding lack of sensitivity. Thus, because of the high costs, risk of accidental infection with live virus and the lower than desired levels of sensitivity of

the previously available tests, there exists a need for rapid, simple and highly sensitive diagnostic tests for detecting flavivirus infection and/or contamination.

The present invention meets these needs by providing highly immunogenic recombinant antigens for use in diagnostic assays for the detection of antibodies to
5 selected flaviviruses. The present invention further provides for the use of recombinant antigens derived from flaviviruses, flavivirus genes or mimetics thereof in immunodiagnostic assays for the detection of antibodies to flavivirus proteins.

Summary of the Invention

The present invention provides a nucleic acid molecule which contains a
10 transcriptional unit (TU) for an immunogenic flavivirus antigen. The TU directs a host cell, after being incorporated within the cell, to synthesize the antigen. In an important aspect of the invention, the flavivirus can be yellow fever virus (YFV), dengue serotype 1 virus (DEN-1), dengue serotype 2 virus (DEN-2), dengue serotype 3 virus (DEN-3), dengue serotype 4 virus (DEN-4), St. Louis encephalitis virus (SLEV), Japanese
15 encephalitis virus (JEV), West Nile virus (WNV), Powassan virus or any other flavivirus. In important embodiments of the present invention, the antigen can be the flavivirus prM/M protein, the E protein, or both. In particular, when the TU includes both the prM/M and E proteins, the host cell secretes subviral particles containing the prM/M and E antigens. In a further important aspect of the invention, the nucleic acid
20 is a DNA molecule. In additional significant embodiments, the nucleic acid TU includes a control sequence disposed appropriately such that it operably controls the expression of the prM/M and E antigens and this control sequence can be the cytomegalovirus immediate early promoter. In an additional embodiment, the nucleotide sequence of the TU is engineered to optimize eukaryotic translation by
25 minimizing large hairpin structures in the 5'-end untranslated region of an mRNA produced by the TU and/or the inclusion of a Kozak consensus sequence at the

0922615-040404

translational start site of an mRNA produced by the TU. In an additional embodiment, the transcriptional unit also includes a poly-A terminator.

5 The present invention further provides a host cell comprising a nucleic acid molecule which includes a transcriptional unit for an immunogenic flavivirus antigen that directs the host cell to synthesize the immunogenic antigen. The flavivirus may be YFV, DEN-1, DEN-2, DEN-3, DEN-4, SLEV, JEV, WNV, Powassan virus or other flavivirus. In important embodiments, the antigen may be the prM/M protein, the E protein, or both the prM/M and the E proteins. In the latter case, the cell secretes subviral particles containing the prM/M and E antigens.

10 Additionally, the invention provides a composition for vaccinating a subject against a flavivirus containing a nucleic acid molecule that includes a transcriptional unit for an immunogenic flaviviral antigen. The transcriptional unit directs a cell within the body of the subject, after being incorporated therein, to synthesize the immunogenic antigen. The composition further includes a pharmaceutically acceptable
15 carrier. In significant embodiments, the flavivirus may be YFV, DEN-1, DEN-2, DEN-3, DEN-4, SLEV, JEV, WNV, Powassan virus or other flavivirus. Furthermore, the antigen may be the prM/M protein, the E protein, or both the prM/M and the E proteins. In the latter instance, the cell secretes subviral particles comprising the flavivirus prM/M and E antigens. These subviral particles are also referred to as noninfectious
20 recombinant antigen (NRA). In important embodiments, the nucleic acid molecule is a DNA molecule. In further significant embodiments, the transcriptional unit additionally contains a control sequence disposed appropriately such that it operably controls the synthesis of the prM/M and E antigens when the nucleic acid is introduced into the cell of the subject. This control sequence can be the cytomegalovirus
25 immediate early promoter. In a still further embodiment, the transcriptional unit can also include a poly-A terminator.

098615-040404

092615-040401

The invention provides still further a method of immunizing a subject against infection by a flavivirus. The method involves administering to the subject an effective amount of a vaccinating composition that contains a nucleic acid molecule which includes a transcriptional unit for an immunogenic flavivirus antigen. The transcriptional unit directs a cell within the body of the subject, after being taken up by the cell, to synthesize the immunogenic antigen. The composition additionally includes a pharmaceutically acceptable carrier. In significant embodiments of the method, the flavivirus may be YFV, DEN-1, DEN-2, DEN-3, DEN-4, SLEV, JEV, WNV, Powassan virus or other flavivirus. In yet other important aspects of the method, the antigen may be the prM/M protein, the E protein, or both the prM/M and the E proteins. When the antigen is both the prM/M and the E proteins, the cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the flaviviral prM/M and E antigens. Additionally, in significant embodiments of the method, the vaccinating composition is administered to the subject in a single dose, via a parenteral route. In yet a further aspect of the method, the nucleic acid is a DNA molecule. In yet additional embodiments of the method, the transcriptional unit further includes a control sequence disposed appropriately such that it operably controls the synthesis of the prM/M and E antigens and in a significant aspect of this embodiment, the control sequence is the cytomegalovirus immediate early promoter. Furthermore, the transcriptional unit may include a poly-A terminator.

These aspects and embodiments of the invention are the basis for its distinct attributes and advantages. Being a nucleic acid construct involving only portions of the flavivirus genome rather than the sequence encompassing the complete genome, the nucleic acid TU-containing vaccine is completely nonviable. It therefore poses no danger of infection by the flavivirus to those involved in its manufacture or to subjects receiving the vaccine. The nucleic acid vaccine is easy to prepare and easy to administer and is stable in storage prior to use. Unexpectedly it has been found that the nucleic acid vaccine of the invention is essentially 100% successful in conferring protective immunity in mammals after administering only a single dose. A further

5

10

20

25

not react with antibodies in human serum. An example of such a carrier is bovine serum albumin (BSA).

The antigens of the present invention can also be recombinant proteins obtained by expressing nucleic acids encoding the antigen in an expression system capable of
5 producing the antigen.

The amino acid sequences of the present antigens can contain an immunoreactive portion of the prM, M and/or E antigen. These antigens may further be attached to sequences designed to provide for some additional property, such as to remove/add amino acids capable of disulfide bonding to increase the reactivity of an
10 epitope by providing a more rigid secondary structure, to increase its bio-longevity or to alter its cytotoxicity or to prevent infection. In any case, the antigen must possess immunoreactivity and/or immunogenicity.

Brief Description of the Drawings

Figure 1 is a schematic representation of flaviviral polyprotein processing. The
15 central horizontal region provides a schematic representation of the viral genome. The lines denote the 5' and 3' non-translated regions and the boxed regions represent the open reading frame for structural (left and top) and non-structural (right and bottom) proteins. Cleavage by host cell signalase occurs simultaneously with translation at the E protein C-terminus, separating structural and non-structural regions. A subtilase-like
20 cellular enzyme, furin, may be responsible for prM cleavage. Potential transmembrane domains of viral polyprotein are indicated by shaded areas.

Figure 2 is a map of the JEV genome (top) and the DNA sequence of oligonucleotides used in a reverse transcriptase-polymerase chain reaction (RT-PCR) (center) to construct the transcription unit for the expression of prM-E protein coding
25 regions (bottom). Potential transmembrane domains of viral polyprotein are indicated by shaded areas.

000015-04000
F04070-5F52360

Figure 3 shows a schematic representation of the plasmid vectors, pCDNA3, pCBamp, and pCIBamp, and the relationship between them. These plasmids include the CMV (cytomegalovirus) promoter/enhancer element, BGHp(A) (bovine growth hormone polyadenylation signal and transcription termination sequence), ampicillin resistance gene and ColE1 origin of replication for selection and maintenance in *E. coli*. The fl origin of replication for single-stranded rescue in *E. coli* cells, SV40 origin of replication (SV40 ORI), neomycin resistance coding region and SV40p(A) sequences were deleted from pCDNA3 to generate pCBamp. An intron sequence was inserted in the NcoI-KpnI site of pCBamp to generate plasmid pCIBamp.

Figure 4 shows SDS-PAGE-immunoblot analyses of the sucrose gradient purified subviral particles from JE-4B COS-1 culture fluid (4B, right lane of each pair). The density gradient purified JE virion from JEV infected C6/36 cell culture was used as a positive control (JEV, left lane of each pair). JE HIAF (hyperimmune ascitic fluid); 4G2, anti-E monoclonal antibody; JM01, anti-M monoclonal antibody; NMAF (normal mouse ascitic fluid).

Figure 5 shows a profile of the E antigen in a rate zonal sucrose gradient analysis prepared from the PEG precipitate of JE-4B cell culture medium with or without Triton X-100 treatment.

Detailed Description of the Invention

The invention encompasses nucleic acid transcriptional units which encode flaviviral antigenic proteins, such as the prM/M and E protein antigens. The nucleic acids function to express the prM/M and E protein antigens when the nucleic acid is taken up by an appropriate cell, especially when the cell is the cell of a subject. The invention also encompasses a vaccine whose active agent is the nucleic acid transcriptional unit (TU). The invention further encompasses cells containing a TU. The invention in addition encompasses a method of immunizing a subject against

flaviviral infection by administering to the subject an effective amount of a vaccine containing the nucleic acid TU molecules.

09826115-040401
The invention provides an isolated nucleic acid comprising a transcriptional unit encoding a signal sequence of a structural protein of a first flavivirus and an
5 immunogenic flavivirus antigen of a second flavivirus, wherein the transcriptional unit directs the synthesis of the antigen. The invention further encompasses the use of the nucleic acid transcriptional unit (TU) to generate flaviviral antigens and the flaviviral antigens produced by the nucleic acid TU. The invention still further encompasses the use of the flaviviral antigens encoded by the TU of the invention to produce flavivirus-
10 specific antibodies and to detect the presence of flavivirus-specific antibodies.

In one embodiment, the isolated nucleic acid of this invention can comprise a transcriptional unit encoding a Japanese encephalitis virus signal sequence.

In another embodiment, the transcriptional unit of this invention can encode an immunogenic flavivirus antigen which can be from one or more of the following
15 flaviviruses: yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

In a particular embodiment, the nucleic acid of this invention can encode a signal sequence of Japanese encephalitis virus and an M protein and an E protein of
20 West Nile virus, SLEV, YFV and/or Powassan virus. The nucleic acid can also encode an immunogenic antigen which can be an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and/or both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus. In a preferred
25 embodiment, the isolated nucleic acid encodes both the M protein and the E protein of the flavivirus. Further, the nucleic acid of the invention can be DNA and can comprise

nucleotide sequence SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 or SEQ ID NO:23.

The transcriptional unit of this invention can also comprise a control sequence disposed appropriately so that it operably controls the synthesis of the antigen. The control sequence can be, for example, the cytomegalovirus immediate early promoter. The nucleic acid of this invention can also comprise a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the transcriptional unit. The transcriptional unit of this invention can also comprise a poly-A terminator.

10 The present invention further provides a cell comprising the nucleic acid of this invention.

Also provided is a composition comprising a pharmaceutically acceptable carrier and nucleic acid or cell or antigen of this invention. The present invention additionally provides a method of immunizing a subject against infection by a
15 flavivirus, comprising administering to the subject an effective amount of a composition of this invention. In a particular embodiment, the composition used to immunize a subject directs the synthesis of both the M protein and the E protein of a flavivirus and a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.
20 Alternatively, the composition can comprise an M protein and/or E protein of a flavivirus or subviral particles comprising the M protein and E protein. In the methods of this invention, the immunizing composition can be administered to the subject in a single dose and can be administered via a parenteral route.

This invention further provides the antigens produced from the isolated nucleic acids of this invention. As an example, the antigen from the second flavivirus encoded by the nucleotide sequence of TU can be the M protein which can be, for example, from

TECHNO-QUEST

proteins. The TU has biological activity such that, after having been introduced into a suitable cell, the nucleic acid induces the synthesis of one or more specified gene products encoded by the nucleic acid. The gene product(s) is(are) other biological macromolecules, such as proteins, not chemically related to the TU. The nucleic acid

5 TU induces the cell to employ its cellular components to produce the specific gene product or products encoded by the nucleic acid of the TU. Although any nucleic acid may serve as a TU, in a preferred embodiment, the TU is the DNA of a plasmid or similar vector, wherein the plasmid or vector comprises coding sequences of marker genes or other sequence constructions that facilitate use of the TU for experimentation

10 and biosynthesis.

As used herein, a "control sequence" is a regulatory nucleotide sequence incorporated within a TU which interacts with appropriate cellular components of the cell and leads to enhanced or activated biosynthesis of the gene products encoded by

15 the TU. Thus a suitable control sequence is one with which the components of the cell have the capability to interact, resulting in synthesis of the gene product. When operably disposed in a nucleic acid with respect to a specified coding sequence, a control sequence effectively controls expression of the specified nucleic acid to produce the gene product.

20 As used herein, a "promoter" is a nucleotide sequence in a TU which serves as a control sequence.

As used herein, a "Kozak sequence" or "Kozak consensus sequence" is a nucleotide sequence at the translational start site which optimizes translation of eukaryotic mRNAs (Kozak, *Mol. Cell. Biology* 9: 5134-5142 (1989)).

25 As used herein, a "terminator" is an extended nucleotide sequence which acts to induce polyadenylation at the 3' end of a mature mRNA. A terminator sequence is found after, or downstream from, a particular coding sequence.

As used herein, a "cell" is a prokaryotic or eukaryotic cell comprising a TU coding for one or more gene products, or into which such a TU has been introduced. Thus, a cell harbors a foreign or heterologous substance, the TU, which is not naturally or endogenously found in the cell as a component. A suitable cell is one which has the capability for the biosynthesis of the gene products as a consequence of the introduction of the TU. In particular, a suitable cell is one which responds to a control sequence and to a terminator sequence, if any, that may be included within the TU. In important embodiments of the present invention, the cell is a mammalian cell. In particularly important embodiments of this invention, the cell is a naturally occurring cell in the body of a human or nonhuman subject to whom (which) the TU has been administered as a component of a vaccine. Alternatively, in analytical, or diagnostic applications, including preparation of antigen for use as a vaccine or in immunodiagnostic assays, or for demonstrative purposes, the cell may be a human or nonhuman cell cultured *in vitro*.

As used herein, a "vaccine" or a "composition for vaccinating a subject" specific for a particular pathogen means a preparation, which, when administered to a subject, leads to an immunogenic response in a subject. As used herein, an "immunogenic" response is one that confers upon the subject protective immunity against the pathogen. Without wishing to be bound by theory, it is believed that an immunogenic response may arise from the generation of neutralizing antibodies (i.e., a humoral immune response) or from cytotoxic cells of the immune system (i.e., a cellular immune response) or both. As used herein, an "immunogenic antigen" is an antigen which induces an immunogenic response when it is introduced into a subject, or when it is synthesized within the cells of a host or a subject. As used herein, an "effective amount" of a vaccine or vaccinating composition is an amount which, when administered to a subject, is sufficient to confer protective immunity upon the subject. Historically, a vaccine has been understood to contain as an active principle one or more specific molecular components or structures which comprise the pathogen, especially its surface. Such structures may include surface components such as

proteins, complex carbohydrates, and/or complex lipids which commonly are found in pathogenic organisms.

As used herein, however, it is to be stressed that the terms "vaccine" or "composition for vaccinating a subject" extend the conventional meaning summarized in the preceding paragraph. As used herein, these terms also relate to the TU of the instant invention or to compositions containing the TU. The TU induces the biosynthesis of one or more specified gene products encoded by the TU within the cells of the subject, wherein the gene products are specified antigens of a pathogen. The biosynthetic antigens then serve as an immunogen. As already noted, the TU, and hence the vaccine, may be any nucleic acid that encodes the specified immunogenic antigens. In a preferred embodiment of this invention, the TU of the vaccine is DNA. The TU can include a plasmid or vector incorporating additional genes or particular sequences for the convenience of the skilled worker in the fields of molecular biology, cell biology and viral immunology (See Molecular Cloning: A Laboratory Manual, 2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and Current Protocols in Molecular Biology, Ausubel et al., John Wiley and Sons, New York 1987 (updated quarterly), which are incorporated herein by reference).

The TU molecules of the instant invention comprise nucleic acids, or derivatives of nucleic acids, having nucleotide sequences that encode specific gene products related to antigens of flaviviruses such as, but not limited to, WNV, JEV, dengue virus, yellow fever virus and SLEV. Although any nucleic acid may serve as a TU, in an important embodiment, the TU is DNA. Alternatively, the nucleic acids may be RNA molecules. They may also be any one of several derivatives of DNA or RNA having a backbone of phosphodiester bonds that have been chemically modified to increase the stability of the TU as a pharmaceutical agent. Modifications so envisioned include, but are not limited to, phosphorothioate derivatives or phosphonate derivatives.

The genome of JEV has been characterized and sequenced (Figures 1 and 2). The M structural protein is expressed as a portion of the polyprotein which includes a pre-M sequence (pr). This pr sequence, immediately amino terminal to the M protein sequence, prevents conformational problems in the processing of the polyprotein. In particular, the presence of the pr sequence is important in preventing misfolding of the E protein. Thus, the presence of prM allows for assembly of JEV particles. Once the virion or particle is formed, the pr sequence can be cleaved from the prM protein to yield mature virus particles containing M proteins, although cleavage of the prM protein to yield M protein is not necessary to produce infectious particles. The prM sequences from many different, related flaviviruses are cleaved to but a low extent, but the flaviviruses themselves are nonetheless, infectious. Examples of such related flaviviruses with similar genomic structures and functions include, but are not limited to WNV, YFV, dengue virus and SLEV.

In one embodiment, the TU encoding flaviviral M and E proteins in the instant invention is DNA. In accord with the discussion in the preceding paragraph, this DNA comprises a nucleotide sequence which encodes the M protein, comprising the pre-M sequence, and a nucleotide sequence encoding the E protein. In this way, the intended gene products are enabled to form subviral particles within the cell. The pre-M sequence can then be cleaved in a fashion analogous to that which occurs with respect to replete virions.

In order to function effectively *in vivo* as a vaccine, it is advantageous to include within the TU a control sequence that has the effect of enhancing or promoting the transcription of the nucleotide sequences encoding the antigens. Use of such promoters is well known to those of skill in the fields of molecular biology, cell biology and viral immunology (See Molecular Cloning: A Laboratory Manual, 2nd Ed., Sambrook,

Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; and Current Protocols in Molecular Biology, Ausubel et al., John Wiley and Sons, New York 1987 (updated quarterly)). When the TU is used as a vaccine in a mammalian host, the promoter to be employed is preferably one which operates effectively in mammalian cells. Such a promoter is disposed with respect to the coding sequences from which transcription is to be promoted, at a position at which it may operably promote such transcription. In a significant embodiment of the instant invention, this promoter is the cytomegalovirus early promoter. In addition, in a further preferred embodiment of the invention, the coding sequences are followed, in the TU nucleic acid, by a terminator sequence (Sambrook et al.). Particular embodiments of the invention relate to both prokaryotic and eukaryotic cells. Many promoter sequences are known that are useful in either prokaryotic or eukaryotic cells. (See Sambrook et al.)

The nucleic acids of the invention may further include DNA sequences known to those of skill in the art to act as immunostimulatory elements. Examples of such elements include, but are not limited to, certain CpG motifs in bacterial DNA (Sato et al., *Science* 273: 352-354 (1996); Klinman et al., *Vaccine* 17: 19-25 (1998)).

Preparation of the TU of the invention is readily accomplished by methods well known to workers of skill in the field of molecular biology. Procedures involved are set forth, for example, in Molecular Cloning: A Laboratory Manual, 2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989 and Current Protocols in Molecular Biology, Ausubel et al., John Wiley and Sons, New York 1987 (updated quarterly). The flaviviral RNA molecule may be isolated from a sample of live virus by methods widely known among virologists familiar with flaviviruses, for example, and with other groups of viruses as well. Methods used with JEV are summarized in Kuno et al. (*J. Virol.* 72: 73-83 (1998)). The RNA is used as a template for the synthesis of cDNA using reverse transcriptase. From the cDNA, a fragment containing the pre-M through E coding region (Figure 2) is obtained by digestion with restriction nucleases known to cleave the cDNA

appropriately to provide such fragments. Examples of restriction digestion of JEV are provided in Nitayaphan et al. (1990) and Konishi et al. (1991). Incorporation of promoters, such as the cytomegalovirus promoter, sequences to promote efficient translation, such as the Kozak sequence, and of the polyadenylation signal, is likewise well known to skilled practitioners in molecular biology and recombinant DNA engineering (Kozak, *Mol. Cell. Biology* 9: 5134-5142 (1989); Azevedo et al., *Braz. J. Med. Biol. Res.* 32: 147-153 (1999)). When a nucleic acid comprising a TU containing the desired coding sequences and control sequences is prepared, it may be obtained in larger quantities by methods that amplify nucleic acids. Such methods are widely known to workers skilled in molecular biology and recombinant DNA engineering. Examples of these methods include incorporation of the nucleic acid into a plasmid for replication by culturing in a cell such as a prokaryotic cell and harvesting the plasmid after completing the culture, as well as amplification of the nucleic acid by methods such as PCR and other amplification protocols, as are well known in the art. These examples are not intended to limit the ways in which the nucleic acid containing the TU may be obtained.

The TU-containing nucleic acid molecules of the instant invention may be introduced into appropriate cells in many ways well known to skilled workers in the fields of molecular biology and viral immunology. By way of example, these include, but are not limited to, incorporation into a plasmid or similar nucleic acid vector which is taken up by the cells, or encapsulation within vesicular lipid structures such as liposomes, especially liposomes comprising cationic lipids, or adsorption to particles that are incorporated into the cell by endocytosis.

In general, a cell of this invention is a prokaryotic or eukaryotic cell comprising a TU, or into which a TU has been introduced. The TU of the present invention induces the intracellular biosynthesis of the encoded prM/M and E antigens. A suitable cell is one which has the capability for the biosynthesis of the gene products as a consequence of the introduction of the nucleic acid. In particular embodiments of the

invention, a suitable cell is one which responds to a control sequence and to a terminator sequence, if any, which may be included within the TU. In order to respond in this fashion, such a cell contains within it components which interact with a control sequence and with a terminator and act to carry out the respective promoting and terminating functions. When the cell is cultured *in vitro*, it may be a prokaryote, a single-cell eukaryote or a multicellular eukaryote cell. In particular embodiments of the present invention, the cell is a mammalian cell. In these cases, the synthesized prM/M and E protein gene products are available for use in analytical, or diagnostic applications, including preparation of antigen for use as a vaccine or in immunodiagnostic assays, or for demonstrative purposes.

In some circumstances, such as when the cell is a cultured mammalian cell, the prM/M and E antigens are secreted in the form of subviral particles. These are aggregates of prM/M and E proteins resembling live virus in surface ultrastructural morphology and immunogenic properties. Since the TU of the invention does not include the remainder of the flaviviral genome, however, there is no capsid incorporated, and most importantly, no infectious viral RNA.

In another important embodiment of this invention, the cell is a natural cellular component of the subject to whom the TU has been administered as a vaccine. The TU, when administered to the subject, is taken up by the cells of the subject. The subject's cells have the capability of responding to any promoter sequences, and terminator, if present. In any case, the TU induces the subject's cells to synthesize flaviviral prM/M and E gene products. Without wishing to be constrained by theoretical considerations, it is believed that the subject's cells produce subviral particles *in vivo* consisting of the prM/M and E antigens, just as has been found to occur with cultured mammalian cells *in vitro*. Such subviral particles, it is believed, then serve as the *in vivo* immunogen, stimulating the immune system of the subject to generate immunological responses which confer protective immunity on the subject. Again without wishing to be limited by theory, the resulting protective immunity may

arise via either humoral or cellular immunity, i.e., via either an MHC class II- or class I-restricted mechanism, respectively, or by both mechanisms.

According to the invention, subjects are immunized against infection by flaviviruses, such as JEV, YFV, dengue virus, SLEV, WNV or other flaviviruses by
5 administering to them an effective amount of a TU comprising nucleic acid which encodes the prM and/or E antigens. The nucleic acid, after being incorporated into the cells of the subject, leads to the synthesis of the flaviviral prM/M and/or E antigens.

In order to administer the TU to the subject, it is incorporated into a composition which comprises a pharmaceutically acceptable carrier. The term
10 "pharmaceutically acceptable" means a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an subject along with the immunogenic material (i.e., recombinant flavivirus protein antigens or portions thereof) without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the vaccine in which it is contained. Examples of
15 pharmaceutically acceptable carriers, or components thereof, include water, physiological saline and common physiological buffers (for further examples, see Arnon, R. (Ed.) Synthetic Vaccines I: pp. 83-92, CRC Press, Inc., Boca Raton, Florida, 1987).

20 It is understood by those skilled in the art that the critical value in describing a vaccination dose is the total amount of immunogen needed to elicit a protective response in a host which is subject to infectious disease caused by virulent or wild-type flavivirus infection. The number and volume of doses used can be varied and are determined by the practitioner based on such parameters as, age, weight, gender,
25 species, type of vaccine to be administered, mode of administration, overall condition of the subject, et cetera, as well as other important factors recognized by those of skill in the art.

5 The TU may be administered to a subject orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, intranasally, topically or the like. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the TU
10 required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the immunogenicity of the vaccine used, the strain or species of flavivirus against which the subject is being immunized, the mode of administration and the like. Thus, it is not possible to specify an exact amount for every embodiment of the present invention. However, an appropriate amount can be
15 determined by one of ordinary skill in the art using only routine experimentation given the teachings herein and what is available in the art.

15 Parenteral administration of the vaccine of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

20 For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound as described herein and optional
25 pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate,

25 In general, in order to immunize a subject against infection by WNV, JEV, YFV, dengue virus, SLEV, or other flaviviruses according to the present invention, and

recognizing that the TUs employed in such methods may have differing overall sizes, doses ranging from about 0.1 µg/kg body weight to about 50 µg/kg body weight can be used.

It has unexpectedly been found that a TU of the present invention which is a
5 DNA confers protective immunity at a level of effectiveness approximating 100% after administration of only a single effective dose of the TU by i.m. injection or by electrotransfer. This is in contrast to many immunization methods carried out using conventional vaccines (as described above), which require one or more booster vaccinations and which may not confer protective immunity to an effectiveness near
10 100%.

It has further been found unexpectedly that protective immunity may be transmitted from a vaccinated female subject to the offspring of the subject. A significant proportion of neonatal mice was shown to be protected against viral challenge after the mothers were vaccinated using the TU DNA of the invention.
15 Without wishing to be limited by theory, it is known that passive immunity may be conferred on neonatal mammals due to the presence in maternal milk of neutralizing antibodies specific for various pathogens. It is possible that the protective immunity against JEV found within the neonates was transmitted to them in this way.

In another embodiment of the invention, the TU encodes a signal sequence of a
20 structural protein of a first flavivirus and an immunogenic flavivirus antigen of a second flavivirus. Thus, in one embodiment, for example, the signal sequence of structural protein of a first flavivirus is replaced by a signal sequence of structural protein of a second flavivirus, which results in proper folding of the nascent polypeptide, proper processing in a host, and/or proper folding of the processed protein.
25 In another embodiment of the invention, the TU may encode an immunogenic flavivirus antigen wherein the antigen comprises sequence from one or more than one flavivirus.

000015-0400
F000000-51192860

032215-04041
T04040-5132350

The present invention further provides immunogenic compositions comprising the polypeptides of this invention in a pharmaceutical acceptable carrier for use as a protein vaccine. Antigens produced from the transcriptional units of the present invention can be used to elicit effective immune responses in a subject. Antigens for this purpose can comprise flavivirus prM protein, flavivirus M protein, flavivirus E protein or any combination thereof, including immunogenic fragments of the proteins. A particularly preferred embodiment is the use of the NRA described herein. A further preferred embodiment is a chimeric protein comprising the signal sequence of one flavivirus and the structural protein(s) of one or more different flaviviruses. In a particularly preferred embodiment, the signal sequence of the antigen is the Japanese encephalitis virus signal sequence.

In other embodiments, the protein vaccine of this invention further comprises a suitable adjuvant. As used herein, an "adjuvant" is a potentiator or enhancer of the immune response. The term "suitable" is meant to include any substance which can be used in combination with the vaccine immunogen (i.e., flavivirus prM protein, flavivirus M protein, flavivirus E protein, or any combination thereof) to augment the immune response, without producing adverse reactions in the vaccinated subject. Effective amounts of a specific adjuvant may be readily determined so as to optimize the potentiation effect of the adjuvant on the immune response of a vaccinated subject. In a preferred embodiment, adjuvanting of the vaccines of this invention is a 2 - stage process, utilizing first a 2% aluminum hydroxide solution and then a mineral oil. In specific embodiments, suitable adjuvants can be chosen from the following group: mineral, vegetable or fish oil with water emulsions, incomplete Freund's adjuvant, *E. coli* J5, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, certain synthetic polymers such as Carbopol (BF Goodrich Company, Cleveland, Ohio), poly-amino acids and co-polymers of amino acids, saponin, carrageenan, REGRESSIN (Vetrepharm, Athens, GA), AVRIDINE (N, N-dioctadecyl-N',N'-bis(2-hydroxyethyl)-propanediamine), long chain polydispersed β (1,4) linked mannan polymers interspersed with O-acetylated groups (e.g. ACEMANNAN), deproteinized highly

The invention further provides an antibody produced in response to immunization by the antigen of this invention. The antibodies of the present invention can include polyclonal and monoclonal antibodies which can be intact immunoglobulin molecules, chimeric immunoglobulin molecules, "humanized antibodies," or Fab or F(ab')₂ fragments. Such antibodies and antibody fragments can be produced by techniques well known in the art which include those described in Harlow and Lane (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) and Kohler et al. (*Nature* 256:495-97, 1975) and U.S. Patents

5,545,806, 5,569,825 and 5,625,126, incorporated herein by reference. The antibodies can be of any isotype IgG, IgA, IgD, IgE and IgM.

The present invention can also include single chain antibodies (ScFv), comprising linked V_H and V_L domains and which retain the conformation and specific binding activity of the native idiotype of the antibody. Such single chain antibodies are well known in the art and can be produced by standard methods. (see, e.g., Alvarez et al., *Hum. Gene Ther.* 8: 229-242 (1997)).

Antibodies can be produced against the antigens of this invention which are synthesized from nucleic acid sequences encoding immunogenic amino acid sequences of the prM, M and/or E antigens of one or more flaviviruses and the signal sequence of a different flavivirus (e.g., JEV). Immunogenic peptides synthesized from the use of these chimeric constructs can easily be identified by use of methods well known in the art for identifying immunogenic regions in an amino acid sequence and used to produce the antibodies of this invention.

15 Conditions whereby an antigen/antibody complex can form, as well as assays
for the detection of the formation of an antigen/antibody complex and quantitation of
the detected protein, are standard in the art. Such assays can include, but are not
limited to, Western blotting, immunoprecipitation, immunofluorescence,
immunocytochemistry, immunohistochemistry, fluorescence activated cell sorting
20 (FACS), fluorescence *in situ* hybridization (FISH), immunomagnetic assays, ELISA,
ELISPOT (Coligan et al., eds. 1995. Current Protocols in Immunology. Wiley, New
York.), agglutination assays, flocculation assays, cell panning, etc., as are well known
to the artisan.

As used herein, the term "bind" means the well characterized binding of
25 antibody to antigen as well as other nonrandom association with an antigen.
"Specifically bind" as used herein describes an antibody or other ligand that does not

The antibody or ligand of this invention can be bound to a substrate (e.g., beads, tubes, slides, plates, nitrocellulose sheets, etc.) or conjugated with a detectable moiety or both bound and conjugated. The detectable moieties contemplated for the present invention can include, but are not limited to, an immunofluorescent moiety (e.g., fluorescein, rhodamine), a radioactive moiety (e.g., ^{32}P , ^{125}I , ^{35}S), an enzyme moiety (e.g., horseradish peroxidase, alkaline phosphatase), a colloidal gold moiety and a biotin moiety. Such conjugation techniques are standard in the art (for example, Harlow and Lane, Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989); Yang et al., *Nature* 382: 319-324 (1996)).

The present invention further provides a method of detecting flavivirus antibody in a sample, comprising contacting the sample with the flavivirus antigen of the present invention, under conditions whereby an antigen/antibody complex can form; and
15 detecting formation of the complex, thereby detecting flavivirus antibody in the sample.

The present invention further provides a method of detecting flavivirus antigen in a sample, comprising contacting the sample with an antibody of this invention under conditions whereby an antigen/antibody complex can form; and detecting formation of the complex, thereby detecting flavivirus antigen in the sample.

20 The method of detecting flavivirus antigen in a sample can be performed, for example, by contacting a fluid or tissue sample from a subject with an antibody of this invention and detecting binding of the antibody to the antigen. It is contemplated that the antigen will be on an intact flavivirus virion, will be a flavivirus-encoded protein displayed on the surface of a flavivirus-infected cell expressing the antigen, or will be a
25 fragment of the antigen. A fluid sample of this method can comprise any biological fluid which could contain the antigen or a cell containing the antigen, such as

cerebrospinal fluid, blood, bile, plasma, serum, saliva and urine. Other possible examples of body fluids include sputum, mucus and the like.

5 The method of detecting flavivirus antibody in a sample can be performed, for example, by contacting a fluid or tissue sample from a subject with an antigen of this invention and detecting the binding of the antigen to the antibody. A fluid sample of this method can comprise any biological fluid which could contain the antibody, such as cerebrospinal fluid, blood, bile, plasma, serum, saliva and urine. Other possible examples of body fluids include sputum, mucus and the like.

10 Enzyme immunoassays such as immunofluorescence assays (IFA), enzyme linked immunosorbent assays (ELISA) and immunoblotting can be readily adapted to accomplish the detection of flavivirus antibodies according to the methods of this invention. An ELISA method effective for the detection of the antibodies can, for example, be as follows: (1) bind the antigen to a substrate; (2) contact the bound
15 antigen with a fluid or tissue sample containing the antibody; (3) contact the above with a secondary antibody bound to a detectable moiety which is reactive with the bound antibody (e.g., horseradish peroxidase enzyme or alkaline phosphatase enzyme); (4) contact the above with the substrate for the enzyme; (5) contact the above with a color reagent; and (6) observe/measure color change or development.

20 Another immunologic technique that can be useful in the detection of flavivirus antibodies uses monoclonal antibodies (MAbs) for detection of antibodies specifically reactive with flavivirus antigens in a competitive inhibition assay. Briefly, sample is contacted with an antigen of this invention which is bound to a substrate (e.g., an ELISA 96-well plate). Excess sample is thoroughly washed away. A labeled (e.g.,
25 enzyme-linked, fluorescent, radioactive, etc.) monoclonal antibody is then contacted with any previously formed antigen-antibody complexes and the amount of monoclonal antibody binding is measured. The amount of inhibition of monoclonal antibody binding is measured relative to a control (no antibody), allowing for detection and

0986115 04040
FOI0040-57792860

measurement of antibody in the sample. The degree of monoclonal antibody inhibition can be a very specific assay for detecting a particular flavivirus variety or strain, when based on monoclonal antibody binding specificity for a particular variety or strain of flavivirus. MAbs can also be used for direct detection of flavivirus antigens in cells
5 by, for example, immunofluorescence assay (IFA) according to standard methods.

As a further example, a micro-agglutination test can be used to detect the presence of flavivirus antibodies in a sample. Briefly, latex beads, red blood cells or other agglutinable particles are coated with the antigen of this invention and mixed with a sample, such that antibodies in the sample that are specifically reactive with the
10 antigen crosslink with the antigen, causing agglutination. The agglutinated antigen-antibody complexes form a precipitate, visible with the naked eye or measurable by spectrophotometer. In a modification of the above test, antibodies of this invention can be bound to the agglutinable particles and antigen in the sample thereby detected.

The present invention further provides a method of diagnosing a flavivirus
15 infection in a subject, comprising contacting a sample from the subject with the antigen of this invention under conditions whereby an antigen/antibody complex can form; and detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

The present invention further provides a method of diagnosing a flavivirus
20 infection in a subject, comprising contacting a sample from the subject with the antibody of this invention under conditions whereby an antigen/antibody complex can form; and detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

In the diagnostic methods taught herein, the antigen of this invention can be
25 bound to a substrate and contacted with a fluid sample such as blood, serum, urine or saliva. This sample can be taken directly from the patient or in a partially purified

0332U3 14114

form. In this manner, antibodies specific for the antigen (the primary antibody) will specifically react with the bound antigen. Thereafter, a secondary antibody bound to, or labeled with, a detectable moiety can be added to enhance the detection of the primary antibody. Generally, the secondary antibody or other ligand, which is reactive, either
5 specifically with a different epitope of the antigen or nonspecifically with the ligand or reacted antibody, will be selected for its ability to react with multiple sites on the primary antibody. Thus, for example, several molecules of the secondary antibody can react with each primary antibody, making the primary antibody more detectable.

The detectable moiety allows for visual detection of a precipitate or a color
10 change, visual detection by microscopy, or automated detection by spectrometry, radiometric measurement or the like. Examples of detectable moieties include fluorescein and rhodamine (for fluorescence microscopy), horseradish peroxidase (for either light or electron microscopy and biochemical detection), biotin-streptavidin (for light or electron microscopy) and alkaline phosphatase (for biochemical detection by
15 color change).

Particular embodiments of the present invention are set forth in the examples which follow. These examples are not intended to limit the scope of the invention as disclosed in this specification.

Examples

20 General methods utilizing molecular biology and recombinant DNA techniques related to preparing and expressing the nucleic acid TU molecules of the invention are set forth in, for example, Current Protocols in Molecular Biology, Ausubel et al., John Wiley and Sons, New York 1987 (updated quarterly), and Molecular Cloning: A Laboratory Manual 2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor
25 Laboratory, Cold Spring Harbor, NY, 1989.

All vector constructions and analyses were carried out by using standard techniques (Sambrook et al., 1989). RT-PCR amplified cDNA, digested with KpnI and NotI nucleases, was inserted into the KpnI-NotI site of eukaryotic expression plasmid

vector (pCDNA3, Invitrogen, Carlsbad, CA). Electroporation-competent *Escherichia coli* XL1-Blue cells (Stratagene, La Jolla, CA) were transformed by electroporation (Gene Pulser™, Bio-Rad, Hercules, CA) and plated onto LB agar plates containing 100 µg/mL carbenicillin (Sigma Chemical Co., St. Louis, MO). Clones were picked and
5 inoculated into 3 mL LB broth containing 100 µg/mL carbenicillin. Plasmid DNA was extracted from a 14 h culture using a QIAprep™ Spin Miniprep Kit (Qiagen). Automated DNA sequencing was performed as recommended (Applied Biosystems/Perkin Elmer, Foster City, CA). Both strands of the cDNA were sequenced and shown to be identical to the sequence for the original SA14 strain (Nitayaphan et
10 al., 1990).

The fragment of plasmid pCDNA3 (Invitrogen, Carlsbad, CA) from nucleotide (nt) 1289 to nt 3455, containing fl ori, SV40 ori, the neomycin resistance gene, and SV40 poly(A) elements was deleted by PvuII digestion and then ligated to generate the pCBamp plasmid. The vector pCIBamp, containing a chimeric intron insertion at the
15 NcoI/KpnI site of the pCBamp was constructed by excising the intron sequence from pCI (Promega, Madison, WI) by digestion with NcoI and KpnI. The resulting 566-bp fragment was cloned into pCBamp by digesting with NcoI-KpnI to replace its 289-bp fragment. Figure 3 presents the relationships between the plasmids pCDA3, pCBamp, and pCIBamp.

20 Plasmids containing the transcriptional unit encoding JEV prM and E proteins were prepared from these plasmids. The cDNA fragment containing the JEV prM and E coding regions in the recombinant plasmid pCDJE2-7 (nucleotide sequence, SEQ ID NO:10; amino acid sequence, SEQ ID NO:11), derived from the pCDNA3 vector, was excised by digestion with NotI and KpnI or XbaI and cloned into the KpnI-NotI site of
25 pCBamp, pCIBamp, pCEP4 (Invitrogen, Carlsbad, CA), or pREP4 (Invitrogen, Carlsbad, CA), or into the SpeI-NotI site of pRc/RSV (Invitrogen, Carlsbad, CA) expression vector to create pCBJE1-14 (nucleotide sequence, SEQ ID NO:17; amino acid sequence, SEQ ID NO:18), pCIBJES14, pCEJE, pREFE, and pRCJE, respectively.

Both strands of the cDNA from clones of each plasmid were sequenced and recombinant clones with the correct nucleotide sequence were identified. Plasmid DNA for use in the in vitro transformation of mammalian cells or mouse immunization experiments was purified by anion exchange chromatography using an EndoFree™

5 Plasmid Maxi Kit (Qiagen).

Example 2. Evaluation of JEV prM and E proteins expressed by various recombinant plasmids using an indirect immunofluorescent antibody assay. The expression of JEV specific gene products by the various recombinant expression plasmids was evaluated in transiently transfected cell lines of COS-1, COS-7 and SV-T2 (ATCC, Rockville MD; 1650-CRL, 1651-CRL, and 163.1-CCL, respectively) by indirect immunofluorescent antibody assay (IFA). The SV-T2 cell line was excluded from further testing since a preliminary result showed only 1-2% of transformed SV-T2 cells were JEV antigen positive. For transformation, cells were grown to 75% confluence in 150 cm² culture flasks, trypsinized, and resuspended at 4°C in phosphate buffered saline (PBS) to a final cell count 5 x 10⁶ per mL. 10 µg of plasmid DNA was electroporated into 300 µL of cell suspension using a BioRad Gene Pulse™ (Bio-Rad) set at 150 V, 960 µF and 100 Ω resistance. Five minutes after electroporation, cells were diluted with 25 mL fresh medium and seeded into a 75 cm² flask. 48 h after transformation the medium was removed from the cells, and the cells were trypsinized and resuspended in 5 mL PBS with 3% normal goat serum. 10 µL aliquots were spotted onto slides, air dried and fixed with acetone at -20°C for 20 min. IFA was performed with acetone-fixed plasmid-transformed cells using fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Sigma Chemical Co.) and JEV HIAF.

25 To determine the influence of various promoter and poly(A) elements on the JEV prM and E protein expression, COS-1 and COS-7 cell lines were transiently transformed by an equal amount of pCDJE2-7 (SEQ ID NO:10), pCEJE, pREJE, or pRCJE plasmid DNA. JEV antigens were expressed in both cell lines transformed by

all four recombinant plasmids, thus confirming that the CMV or RSV (rous sarcoma virus) promoter and BGH or SV40 poly(A) elements were functionally active.

However, the percentage of transformed cells and the level of JEV antigens expressed, as determined by the number of IFA positive cells and IFA intensity, respectively,

5 differed greatly among the various plasmids (Table 1). A significantly high percentage of COS-1 cells transformed by pCDJE2-7 (SEQ ID NO:10), pCBE1-14 (SEQ ID NO:17) and pCIBES14 expressed the JEV antigens, and the level of the expressed proteins was compatible with JEV-infected cells. Cells transfected with pCEJE, pREJE, or pRCJE vectors, on the other hand, had a low percentage of antigen-
10 expressing cells, as well as a low intensity of fluorescence, indicating weak expression of the antigens.

In order to ascertain whether the enhanced expression of JEV proteins by pCDJE2-7 (SEQ ID NO:10) was influenced by the SV40-encoded eukaryotic origin of replication, the plasmid pCBE1-14 (SEQ ID NO:17) was constructed so that a 2166-
15 bp fragment, containing fl ori, SV40 ori, the neomycin resistance gene and SV40 poly(a) elements from pCDJE2-7, was deleted. A chimeric intron was then inserted into pCBE1-14 to generate pCIBES14. The pCIBES14 plasmid was used to determine if the expression of JEV proteins could be enhanced by the intron sequence. Following transformation, cells harboring both pCBE1-14 and pCIBES14 vectors
20 expressed a level of JEV antigens similar to that observed with pCDJE2-7 (Table 1). This result indicates that expression of JEV prM and E antigens by recombinant vectors is influenced only by the transcriptional regulatory elements. Neither the eukaryotic origin of replication nor the intron sequence enhanced JEV antigen expression in the cells used. Vectors containing the CMV promoter and BGH poly(A) (Figure 3) were
25 selected for further analysis.

Example 3. Selection of an in vitro transformed, stable cell line constitutively expressing JEV specific gene products. COS-1 cells were transformed with 10 µg of pCDJE2-7 DNA by electroporation as described in the previous example. After a 24 hr

000000-04040

incubation in non-selective culture medium, cells were treated with neomycin (0.5 mg/mL, Sigma Chemical Co.). Neomycin-resistant colonies, which became visible after 2-3 weeks, were cloned by limited dilution in neomycin-containing medium.

- Expression of vector-encoded JEV gene products was initially screened by IFA using
5 JEV HIAF. One JEV-IFA positive clone (JE-4B) and one negative clone (JE-5A) were selected for further analysis and maintained in medium containing 200 µg/mL neomycin.

- Authenticity of the JEV E protein expressed by the JE-4B clone was demonstrated by epitope mapping by IFA using a panel of JEV E-specific murine
10 monoclonal antibodies (Mab) (Kimura-Kuroda et al., *J. Virol.* 45: 124-132 (1983); Kimura-Kuroda et al., *J. Gen. Virol.* 67: 2663-2672 (1986); Zhang et al., *J. Med. Virol.* 29: 133-138 (1989); and Roehrig et al., *Virol.* 128: 118-126 (1983)). JEV HIAF and normal mouse serum were used as positive and negative antibody controls, respectively. Four JEV-specific, six flavivirus-subgroup specific, and two flavivirus-group reactive
15 Mabs reacted similarly with the 4B clone or JEV-infected COS-1 cells (Table 2).

Example 4. Antigenic properties and immunological detection of subviral particles secreted by the JE-4B COS-1 cell line.

- a. Preparation of subviral particles. JE-4B COS-1 cells were grown and maintained in medium containing 200 µg/mL of neomycin. The cultured medium was
20 routinely harvested and stored at 4°C, and replenished twice weekly, and the cells were split 1:5 every 7-10 days. Culture medium was clarified by centrifugation at 10,000 rpm for 30 min in a Sorvall F16/250 rotor at 4°C, and centrifuged further for 4 hr at 39,000 rpm in a Sorvall TH641 rotor at 4°C through a 5% sucrose cushion (w/w, prepared with 10 mM Tris HCl, pH 7.5, 100 mM NaCl (TN buffer)). The pellet
25 containing subviral particles was resuspended in TN buffer and stored at 4°C. Alternatively, 7% or 10% PEG-8000 (w/v) was added to the clarified culture medium. The mixture was stirred at 4°C for at least 2 hr, and the precipitated particles were

collected by centrifugation at 10,000 rpm for 30 min. The precipitate was resuspended in TN buffer and stored at 4°C. The subviral particles were purified from both pelleted and PEG-precipitated preparations by rate zonal centrifugation in a 5-25% continuous sucrose gradient in TN at 38,000 rpm at 4°C for 90 min. 1-mL fractions were collected from the top of the gradient, tested by antigen capture ELISA (see below), and the positive fractions loaded onto a 25-50% sucrose gradient in TN. This was centrifuged overnight in an equilibrium density centrifugation at 35,000 rpm at 4°C. 0.9-mL fractions from the equilibrium gradients were collected from the bottom. They were tested by antigen-capture ELISA and assessed for hemagglutination (HA) activity at pH 6.6. An aliquot of 100 µL of each fraction was weighed precisely to determine its density. The ELISA-positive fractions were pooled and pelleted at 39,000 rpm at 4°C for 3-4 hr and the pellet resuspended in TN buffer. Antigen-capture ELISA and HA titers were determined on the pelleted samples. JEV-infected COS-1 cell supernatant was also subjected to similar purification protocols as detailed above and used as a positive control for the gradient analysis. JE virions were also purified from infected C6/36 cells 5-6 days postinfection by sedimentation in a glycerol/tartrate equilibrium gradient.

b. Western blots of subviral particles. Gradient-purified samples of the subviral particles were mixed with electrophoresis sample buffer and run on 10 or 12.5% sodium dodecyl sulfate-containing polyacrylamide gels (SDS-PAGE) as described by Laemmli (*Nature* 277: 680-685 (1970)). Proteins were transferred to a nitrocellulose membrane and immunochemically detected with polyclonal JEV HIAF, flavivirus cross-reactive anti-E Mab 4G2 (Henchal et al., *Amer. J. Trop. Med. Hyg.* 31: 830-836 (1982)), or mouse anti-prM peptide hyperimmune serum (JM01). Figure 4 shows a comparison of the M and E proteins produced by JEV infected C6/36 and JE-4B COS-1 cells. Some nonspecific reactivity to E protein was observed in the normal mouse ascitic fluid and Jmo1 anti-peptide serum. Proteins identical in size to M and E were secreted in the subviral particles and could be detected by E-specific Mab 4G2 and prM-specific JM01 antiserum, respectively.

c. Density gradient detection of JEV subviral particles in culture medium. For ELISA, antigen-capture antibody (4G2) was diluted in 0.1 M sodium carbonate buffer, pH 9.6, and used to coat 96-well microtiter plates (Immulon II, Dynatech, Chantilly, VA) by overnight incubation at 4° C. After blocking with 3% normal goat serum in PBS, two-fold serially-diluted samples were added to the 4G2-coated plate and incubated 1.5 hours at 37°C. Captured antigen was detected by horseradish peroxidase-conjugated 6B6C-1 Mag, and incubated for 1 hour at 37°C. The enzyme activity on the solid phase was then detected with TMB (3,3',5,5'-tetramethylbenzidine)-ELISA (Life Technologies, Grand Island, NY).

- 10 Approximately 500 mL of cell culture medium from 15 X 150 cm² flasks of JE-4B cells was collected four days after cells were seeded. PEG-precipitated subviral particles were resuspended in 2 mL of TN buffer, pH 7.5; a 0.7 mL aliquot of this resuspended pellet was loaded onto a 5-25% sucrose gradient. Triton X-100, which disrupts subviral particles, was added to another 0.7 mL aliquot to a final concentration
- 15 of 0.1% and this was loaded onto a 5-25% sucrose gradient prepared in TN buffer containing 0.1% Triton X-100. A definite opaque band was observed approximately 2.5 cm from the top of the gradient containing Triton X-100, but not in the gradient without detergent. Fractions (1 mL) were collected from top to bottom for each gradient (Figure 5). Each collected fraction was analyzed by antigen capture ELISA.
- 20 Antigen was detected in fractions 4-6, indicating relatively rapid sedimentation characteristic of subviral particles. Treatment of the PEG precipitate from JE-4B culture medium with Triton X-100 shifted the position of ELISA-reactive material to the top of the gradient. Thus treatment with Triton X-100 produces only slow-sedimenting molecules. A similar finding was reported by Konishi et al. (*Virology* 188: 714-720 (1992)). These results show that rapidly sedimenting subviral particles
- 25 containing prM/M and E could be disrupted by detergent treatment.

Hemagglutination (HA) activity was determined in the pH range from 6.1 to 7.0 by the method of Clarke and Casals (*Amer. J. Trop. Med. Hyg.* 7: 561-573 (1958)).

The subviral particle secreted by JE-4B cells and the virion particle produced by JEV infected COS-1 cells had a similar HA profile with the optimum pH determined to be 6.6.

Example 5. Comparison of the immune response in mice vaccinated with
5 pCDJE2-7 nucleic acid vaccine of the invention and commercial JEV vaccine. Groups
of five 3-week-old female, ICR outbred mice were injected intramuscularly in the left
and right quadriceps with 100 µg of pCDJE2-7 plasmid in 100 µL of dH₂O or were
given doses of JE-VAX (manufactured by the Research Foundation for Microbial
Disease of Osaka University and distributed by Connaught Laboratories, Swiftwater,
10 PA.) subcutaneously that are one-fifth the dose given to humans. The plasmid
pCDNA3/CAT (Invitrogen), which encodes and expresses an unrelated protein, was
used as the negative vaccination control. Except for one group of pCDJE2-7-
vaccinated mice, all animals were boosted 3 weeks later with an additional dose of
plasmid or JE-VAX. Mice were bled from the retroorbital sinus at 3, 6, 9, 23, 40 and
15 60 weeks after inoculation. JEV antibody titers were determined by enzyme-linked
immunosorbent assay (ELISA) against purified JEV or by plaque reduction neutralization
tests (PRNT) (Roehrig et al., *Viol.* 171: 49-60 (1989); and Hunt and Calisher, *Amer. J.*
Trop. Med. Hyg. 28: 740-749 (1979)).

The pCDJE2-7 nucleic acid vaccine and JE-VAX provided 100%
20 seroconversion within three weeks after the first vaccination in all three groups of mice
(Table 3). The JEV ELISA and PRNT antibody titers reached the highest level at week
6 and week 9, respectively, after immunization. Mice receiving 1 dose of DNA vaccine
had similar antibody responses as those receiving 2 doses. Comparable ELISA
antibody titers were maintained in DNA-vaccinated groups up to 60 weeks, after which
25 the experiment was terminated. However, only one of four mice in the JE-VAX group
was JEV antibody positive at 60 weeks post-inoculation. The pCDNA3/CAT control
group did not have any measurable JEV antibody. These results demonstrate that a

Example 6. Comparison of various nucleic acid vaccine constructs of the invention and commercial JEV vaccine for effectiveness of vaccination at different

Plasmid pCBE1-14 provided the highest extent of seroconversion, i.e., antibody titer greater than 1:1600, achieving 80-100% at both ages of vaccination. Administration of pCDJE2-7 or pCIBJES14 provided moderate seroconversion by 7 weeks when 3-day old mice were vaccinated (60% for each), but weaker seroconversion (40% and 10%, respectively) when measured 3 weeks after vaccination. When these plasmids were administered at the age of 3 weeks, however, seroconversions of 90% or 100% were attained at both 3 weeks and 7 weeks after vaccination. In contrast, the commercial vaccine, JE-VAX, conferred no seroconversion when administered at 3 days of age, and 100% when given at 3 weeks of age. Thus the nucleic acid TU's for JEV prM and E provided an extent of seroconversion better than a very high dose of the commercial vaccine, and unexpectedly high seroconversion in both young and more mature animals.

Example 7. Protective immunity conferred by the nucleic acid vaccine of the invention. Three-day old vaccinated groups from Example 6 were challenged 7 weeks

after vaccination by intraperitoneal injection of 50,000 pfu/100 μ L of the mouse-adapted JEV strain SA14 and observed for 3 weeks. 100% protection was achieved in groups that received various nucleic acid TU-containing vaccine constructs for up to 21 days (Table 5). In contrast, 60% of the JE-VAX-vaccinated mice, as well as 70% of the pCDNA3/CAT-vaccinated negative controls, did not survive virus challenge by 21 days. These results indicate that the nucleic acid TU's of the invention confer unexpectedly effective protection on vaccinated mice. This suggests the possibility of employing the nucleic acid vaccine of the invention as an early childhood vaccine for humans. In contrast, JE-VAX, the inactivated human vaccine currently used, does not appear to be effective in young animals.

Example 8. Passive protection of neonatal mice correlated with the maternal antibody titer. Female ICR mice at the age of 3 weeks were vaccinated with either one dose or two doses spaced two days apart of pCDJE2-7 plasmid DNA, at 100 μ g/100 μ L, or with two doses of JE-VAX that were one-fifth the dose given to humans. The negative control group received two doses of 100 μ g/100 μ L of pCDNA-3/CAT plasmid. Passive protection by maternal antibody was evaluated in pups resulting from matings of experimental females with non-immunized male mice that occurred nine weeks following the first vaccination or 6 weeks following the second vaccination. Pups were challenged between 3-15 days after birth by intraperitoneal administration of 5,000 pfu/100 μ L of mouse-adapted SA14 virus and observed daily for 3 weeks (Table 6). The survival rates correlated with the maternal neutralizing antibody titers. 100% of pups nursed by mothers with a PRNT of 1:80 survived viral infection, whereas none of the pups from the control mother survived (Table 6). Partial protection of 45% and 75% was observed in older pups that were nursed by mothers with a PRNT titer of 1:20 and 1:40, respectively. The survival rates also correlated with the length of time that pups were nursed by the immune mother. As just indicated, 13-15 day old pups had high survival rates. None of the 3-4 day old pups, however, survived virus challenge when the mother had a PRNT titer of 1:20 or 1:40. Thus maternal antibody provides

partial to complete protective immunity to the offspring. In addition, JEV antibody was detected by ELISA in the sera of 97% (29/30) of the post-challenge pups.

Mice were inoculated intramuscularly with 1 or 2, 100 µg doses of plasmid DNA, or subcutaneously with two, 1/5 human doses of JE-VAX vaccine. Sera were collected 9 weeks post-vaccination for PRNT testing prior to mating with non-immune male.

Example 9. Preparation of recombinant plasmids containing the transcriptional unit encoding WNV prM and E antigens. Genomic RNA was extracted from 150 µL of Vero cell culture medium infected with NY 99-6480 strain, an strain isolated from the outbreak in New York 1999, using the QIAamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA). Extracted RNA was eluted and suspended in 80 µl of nuclease-free water, and used as a template for the amplification of WNV prM and E gene coding sequences. Primer sequences were obtained from the work of Lanciotti et al. (*Science* 286: 2333-2337 (1999)). A cDNA fragment containing the genomic nucleotide region was amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR). Restriction sites BsmBI and KasI were engineered at the 5' terminus of the cDNA by using amplicer WN466 (nucleotide sequence, SEQ ID NO:12). An in-frame translation termination codon, followed by a NotI restriction site was introduced at the 3' terminus of the cDNA by using amplicer cWN2444 (SEQ ID NO:13). The RT-PCR product was purified by a QIAquick™ PCR Purification Kit (Qiagen).

The double-stranded amplicon produced by use of the two amplicers above (SEQ ID NO:12 and SEQ ID NO:13) was digested with KasI and NotI enzymes to generate a 998 bp (nt-1470 to 2468) fragment of DNA was inserted into the KasI and NotI sites of a pCBJESS vector to form an intermediate plasmid, pCBINT. The pCBJESS was derived from the pCBamp plasmid, that contained the cytomegalovirus early gene promoter and translational control element and an engineered JE signal

sequence element (Chang et al., *J. Virol.* 74: 4244-4252 (2000)). The JE signal sequence element comprises the JE signal sequence (SEQ ID NO:14).

5 The cDNA amplicon was subsequently digested with BsmBI and Kas I enzymes and the remaining 1003 bp fragment (nt-466 to 1470) was inserted in to the KasI site of pCBINT to form pCBWN (nucleic acid sequence, SEQ ID NO:15; amino acid sequence, SEQ ID NO:16). Automated DNA sequencing using an ABI prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA) was used to confirm that the recombinant plasmid had a correct prM and E sequence as defined by Lanciotti et al. (*Science* 286: 2333-2337 (1999)).

10 Plasmid DNA for use in the in vitro transformation of mammalian cells or mouse immunization experiments was purified by anion exchange chromatography as described in Example 1.

Example 10. Immunochemical characterization and evaluation of WNV prM and E proteins expressed by pCBWN. WNV specific gene products encoded by the pCBWN plasmid were expressed in COS-1 cells. Cells were electroporated and transformed with pCBWN plasmid according to Chang et al. (*J. Virol.* 74: 4244-4252 (2000)). Electroporated cells were seeded onto 75 cm² culture flasks or a 12-well tissue culture dish containing one sterile coverslip/well. All flasks and 12-well plates were kept at 37°C, 5% CO₂ incubator. Forty hours following electroporation, coverslips containing adherent cells were removed from the wells, washed briefly with PBS, fixed with acetone for 2 minutes at room temperature, and allowed to air dry.

15
20

Protein expression was detected using indirect immunofluorescence antibody assay (IFA), as described in Example 2. Flavivirus E-protein specific monoclonal antibody (Mab) 4G2, WNV mouse hyperimmune ascitic fluid (HIAF) and normal mouse serum (NMS) at 1:200 dilution in PBS were used as the primary antibody to detect protein expression (Henchal et al., *Am. J. Trop. Med. Hyg.* 31: 830-836 (1982)).

25

Tissue culture medium was harvested 40 and 80 hours following electroporation. Antigen-capture (Ag-capture) ELISA was used to detect secreted WN virus antigen in the culture medium of transiently transformed COS-1 cells. The Mab 4G2 and horseradish peroxidase-conjugated Mab 6B6C-1 were used to capture the WN virus antigens and detect captured antigen, respectively (Chang et al., *J. Virol.* 74: 4244-4452 (2000); Henchal et al., *Am. J. Trop. Med. Hyg.* 31: 830-836 (1983); Roehrig et al., *Virology* 128: 118-126 (1983)).

WN virus antigen in the medium was concentrated by precipitation with 10 % polyethylene glycol (PEG)-8000. The precipitant was resuspended in TNE buffer (50 mM Tris, 100 mM NaCl, 10 mM EDTA, pH 7.5), clarified by centrifugation, and stored at 4°C. Alternatively, the precipitant was resuspended in a lyophilization buffer (0.1 M TRIZMA and 0.4% bovine serum albumin in borate saline buffer, pH 9.0), lyophilized and stored at 4°C. Lyophilized preparations were used as antigen for the evaluation in MAC- and indirect IgG ELISAs.

WN virus-specific protein was detected by IFA on the transiently transformed COS-1 cells. E, prM and M proteins expressed in these cells were secreted into the culture medium. WN virus antigen concentrated by PEG precipitation was extracted with 7.0 % ethanol to remove residual PEG (Aizawa et al., *Appl. Enviro. Micro.* 39: 54-57 (1980)). Ethanol extracted antigens and gradient-purified WN virions were analyzed on a NuPAGE, 4-12% gradient Bis-Tris Gel in a Excel Plus Electrophoresis Apparatus (Invitrogen Corp., Carlsbad, CA) and followed by electroblotting onto nitrocellulose membranes using a Excel Plus Blot Unit (Invitrogen Corp.). WN virus-specific proteins produced by the transiently transformed COS-1 cells were detected by WN virus specific mouse HIAF or flavivirus E protein reactive Mab 4G2 in a Western blot analysis, using NMS as a negative serum control. The proteins displayed similar reactivity and identical molecular weights to the corresponding gradient purified virion E, prM and M protein derived from WN virus infected suckling mouse brain (SMB).

In analysis of the NRA as an antigen for diagnostic ELISA, one vial of lyophilized NRA, representing antigen harvested from 40 ml of tissue culture fluid, was reconstituted in 1.0 ml of distilled water and compared with the reconstituted WN virus infected suckling mouse brain (SMB) antigen provided as lyophilized as β -

- 5 propiolactone-inactivated sucrose-acetone extracts (Clarke et al., *Am. J. Trop. Med. Hyg.* 7: 561-573 (1958)). All recombinant proteins, prM, M and E, had a similar reactivity to that of the gradient-purified virion E, prM and M proteins.

- 10 Coded human specimens were tested concurrently with antigens in the same test at the developmental stage. The MAC- and IgG ELISA protocols employed were identical to the published methods (Johnson et al., *J. Clin. Microbiol.* 38: 1827-1831 (2000); Martin et al., *J. Clin. Microbiol.* 38: 1823-1826. (2000)). Human serum specimens were obtained from the serum bank in our facility, which consists of specimens sent to the DVVID for WN virus confirmation testing during the 1999 outbreak. In these tests, a screening MAC- and IgG ELISA were performed on a 1:400 specimen dilution. Specimens yielding positive/negative (P/N) OD ratios between 2 and 3 were considered suspect positives. Suspect serum specimens were subject to confirmation as positives by both ELISA end-point titration and plaque-reduction neutralization test (PRNT). All specimens yielding P/N OD ratios greater than 3.0 were considered positives without further confirmatory testing.

- 20 An Ag-capture ELISA employing flavivirus-group reactive, anti-E Mab, 4G2 and 6B6C-1, was used to detect NRA secreted into culture fluid of pCBWN transformed COS-1 cells. The antigen could be detected in the medium one day following transformation; and the maximum ELISA titer (1:32-1:64) in the culture fluid without further concentration was observed between day two and day four. NRA was concentrated by PEG precipitation, resuspended in a lyophilization buffer, and lyophilized for preservation. For diagnostic test development, one vial of lyophilized NRA was reconstituted with 1.0 ml distilled water and titrated in the MAC- or indirect IgG ELISA using WN virus positive and negative reference human sera (Johnson et al.,

The reactivity of NRA and NY-06480, Eg101 and SLE virus SMBs were compared by the MAC- and IgG ELISAs using 21 coded human serum specimens. Of the 21 specimens, 19 had similar results on all three antigens (8 negatives and 11 suspect positives or positives). Eighteen specimens were also tested separately using SLE SMB antigen. Only three of 13 Eg-101-SMB positive specimens were positive in the SLE MAC-ELISA (Table 1). None of WN antigen negative specimens was positive by SLE MAC-ELISA. This result confirmed a previous observation that anti-WN virus IgM did not cross-react significantly with other flaviviruses (Tardei et al., *J. Clin. Microbiol.* 38: 2232-2239 (1940)) and was specific to diagnose acute WN virus infection regardless of whether NRA or SMB antigen was used in the test. All of the specimens were also tested concurrently by indirect IgG ELISA. Ten of 21 specimens were positive using any of the three antigens.

The two discrepant serum specimens (7 and 9) both from the same patient, collected on day-4 and 44 after onset of disease, respectively, were IgM-negative with NRA and SMB NY antigen and IgM-positive using Eg-101 SMB antigen in the initial

test. To investigate these two discordant specimens further, six sequentially collected specimens from this patient were retested by end-point MAC- and IgG ELISAs. A greater than 32-fold serial increase shown in the MAC-ELISA titer between day-3 and day-15 could be demonstrated with all antigens used. Cerebrospinal fluid collected on day-9 after onset of disease also confirmed that this patient indeed was infected by WN shortly prior to taking the sample. The cerebrospinal fluid had IgM P/N reading of 13.71 and 2.04 against Eg-101- and SLE-SMB antigens, respectively. Day-31 and day-44 specimens were negative (<1:400) by using NY-SMB antigen but positive by using NRA and Eg101-SMB. Compatible IgG titers were observed with all three antigen used in the test.

Example 11. Evaluation of the immune response in animals vaccinated with pCBWN. Groups of ten, three-wk-old female ICR mice were used in the study. Mice were injected intramuscularly (i.m.) with a single dose of pCBWN or a green fluorescent protein expressing plasmid (pEGFP) DNA (Clontech, San Francisco, CA.). The pCBWN plasmid DNA was purified from XL-1 blue cells with EndoFree Plasmid Giga Kits (Qiagen) and resuspended in PBS, pH 7.5, at a concentration of 1.0 µg/µl. Mice that received 100 µg of pEGFP were used as unvaccinated controls. Mice were injected with the pCBWN plasmid at a dose of 100, 10, 1.0, or 0.1 µg in a volume of 100 µl. Groups that received 10, 1.0, or 0.1 µg of pCBWN were vaccinated by the electrotransfer mediated *in vivo* gene delivery protocol using the EMC-830 square wave electroporator (Genetronics Inc. San Diego, CA.). The electrotransfer protocol was based on the method of Mir et al., (*Proc. Natl. Acad. Sci. USA* 96: 4262-4267.(1999)). Immediately following DNA injection, transcutaneous electric pulses were applied by two stainless steel plate electrodes, placed 4.5-5.5 mm apart, at each side of the leg. Electrical contact with the leg skin was ensured by completely wetting the leg with PBS. Two sets of four pulses of 40 volts/mm of 25 msec duration with a 200 msec interval between pulses were applied. The polarity of the electrode was reversed between the set of pulses to enhance electrotransfer efficiency.

5 Mice were bled every 3 wks following injection. The WN virus specific antibody response was evaluated by Ag-capture ELISA and plaque reduction neutralization test (PRNT). Individual sera were tested by IgG-ELISA, and pooled sera from 10 mice of each group were assayed by PRNT. All the mice vaccinated with pCBWN had IgG ELISA titers ranging from 1:640 to 1:1280 three wks after vaccination. The pooled sera collected at three and six wks had a Nt antibody titer of 1:80. None of the serum specimens from pEGFP control mice displayed any ELISA or Nt titer to WN virus.

10 To determine if the single i.m. vaccination of pCBWN could protect mice from WN virus infection, mice were challenged with NY-6480 virus either by intraperitoneal injection or by exposure to the bite of virus-infected *Culex* mosquitoes. Half of the mouse groups were challenged intraperitoneally (ip) at 6 wks post vaccination with 1,000 LD₅₀ (1,025 PFU/100 µl) of NY99-6480 virus. The remaining mice were each exposed to the bites of three *Culex tritaeniorhynchus* mosquitoes that has been infected with NY99-6480 virus 7 days prior to the challenge experiment. Mosquitoes were 15 allowed to feed on mice until they were fully engorged. Mice were observed twice daily for three wks after challenge.

20 It was evident that the presence of Nt antibodies correlated with protective immunity, since all mice immunized with WN virus DNA remained healthy after virus challenge while all control mice developed symptoms of CNS infection 4-6 days following virus challenge and died on an average of 6.9 and 7.4 days after intraperitoneal or infective mosquito challenge, respectively. In the vaccinated group, the pooled sera collected three wks after virus challenge (9-wk post immunization) had Nt antibody titers of 1:640 or 1:320. Pooled vaccinated mouse sera reacted only with E 25 protein in the Western blot analysis.

Groups of ten mice were immunized with 10.0 to 0.1 µg of pCBWN per animal by use of electrotransfer. All groups that received pCBWN were completely protected

20101010-51192880

from virus challenge. At 6 wks after immunization all groups of electrotransfer mice had Nt titer less than four-fold different than animals receiving 100 µg of pCBWN by conventional i.m. injection without electrotransfer. Both these results evidencing effective immunization suggest that the electrotransfer protocol enhances the immunogenicity and protective efficacy of the DNA vaccine of the invention (when carried out as described in (Mir et al., *Proc. Natl. Acad. Sci. USA.* 96: 4262-4267.(1999)).

Mixed-bred mares and geldings of various ages used in this study were shown to be WN virus and SLE virus antibody-negative by ELISA and PRNT. Four horses were injected i.m. with a single dose (1,000 µg/1,000 µl in PBS, pH 7.5) of pCBWN plasmid. Serum specimens were collected every other day for 38 days prior to virus challenge, and the WN virus specific antibody response was evaluated by MAC- or IgG ELISA and PRNT.

Two days prior to virus challenge, 12 horses (4 vaccinated and 8 control) were relocated into a bio-safety level (BSL)-3 containment building at the Colorado State University. The eight unvaccinated control horses were the subset of a study that was designed to investigate WN virus induced pathogenesis in horses and the potential of horses to serve as amplifying hosts. Horses were each challenged by the bite of 14 or 15 *Aedes albopictus* mosquitoes that had been infected by NY99-6425 or BC787 virus 12 days prior to horse challenge. Mosquitoes were allowed to feed on horses for a period of 10 min. Horses were examined for signs of disease twice daily. Body temperature was recorded, and serum specimens collected twice daily from days 0 (day of infection) to 10, then once daily through day 14. Pulse and respiration were recorded daily after challenge. The collected serum samples were tested by plaque titration for detection of viremia, and by MAC- or IgG ELISA and PRNT for antibody response.

No systemic or local reaction was observed in any vaccinated horse. Individual horse sera were tested by PRNT. Vaccinated horses developed Nt antibody greater than

14. All unvaccinated control horses became infected with WN virus after exposure to infected mosquito bites. Seven of the eight unvaccinated horses developed viremia that appeared during the first 6 days after virus challenge. Viremic horses developed Nt antibody between day-7 and day-9 after virus challenge. The only horse from the entire study to display clinical signs of disease was horse #11, which became febrile and showed neurologic signs beginning 8 days after infection. This horse progressed to severe clinical disease within 24 hours and was euthanized on day 9. Four representing horses, #9, 10, 14 and 15, presenting viremia for 0, 2, 4, or 6 days, were selected and used as examples in this example. Virus titers ranged from $10^{1.0}$ PFU/ml of serum (in horse #10), the lowest level detectable in our assay, to $10^{2.4}$ /ml (in horse #9). Horse #14 did not develop a detectible viremia during the test period. However, this horse was infected by the virus, as evidenced by Nt antibody detected after day 12.

25 Example 12. Preparation of recombinant plasmids containing coding sequences
for yellow fever virus (YFV) or St. Louis encephalitis virus (SLEV) prM and E
proteins. A strategy similar to constructing the pCDJE2-7 recombinant plasmid was
used to prepare YFV and SLEV recombinant plasmids. Genomic RNA was extracted

from 150 µL of YFV strain TRI-788379 or SLE strain 78V-6507 virus seeds using Q1Aamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA.). The viral RNA was used as a template for amplification of YFV or SLEV prM and E gene coding regions. Primers YFDV389 (nucleotide sequence, SEQ ID NO:4; amino acid sequence, SEQ ID NO:5),
5 cYFDV2452 (SEQ ID NO:6), SLEDV410 (nucleotide sequence, SEQ ID NO:7; amino acid sequence, SEQ ID NO: 8) and cSLEDV2449 (SEQ ID NO:9) were used to generate the corresponding recombinant nucleic acids as described above for the preparation of the JEV and WNV recombinant plasmids. RT-PCR amplified cDNA, digested with KpnI and NotI enzymes, was inserted into the KpnI-NotI site of a
10 eukaryotic expression plasmid vector, pCDNA3 (Invitrogen). Both strands of the cDNA were sequenced and verified for identity to sequences from YFV strain TRI-788379 or SLEV strain 78V-6507. Recombinant plasmids pCDYF2 and pCDSLE4-3, which contained the nucleotide sequences of the prM and E coding regions for YFV or SLEV, respectively, were purified using an EndoFree™ Plasmid Maxi Kit (Qiagen),
15 and used for *in vitro* transformation or mouse immunization.

YFV or SLEV specific antigens were expressed in COS-1 cells transformed by pCDYF2 or pCDSLE4-3, respectively. The level of expressed proteins was similar to a YFV- or SLEV-infected COS-1 cell control. As in the JEV model, COS-1 cell lines transformed by vectors bearing genes for the viral antigens were obtained which
20 constitutively express YFV or SLEV antigenic proteins. Epitope mapping by IFA using a panel of YFV or SLEV E-specific Mabs indicated that the authentic E protein was expressed by the pCDYF2- or pCDSLE4-3-transformed COS-1 cells. A preliminary study indicated that 100% of three week-old female, ICR mice seroconverted after intramuscular inoculation with a single dose of 100 µg/100 µL of pCDSLE4-3 plasmid
25 in deionized water.

Example 13. Preparation of recombinant plasmids containing coding sequences for St. Louis encephalitis virus prM and E antigens with JEV signal sequence.

Genomic RNA was extracted from 150 µL of Vero cell culture medium infected with

MSI-7 strain of St. Louis encephalitis virus using the QIAamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA). Extracted RNA was eluted and suspended in 80 µl of nuclease-free water, and used as a template for the amplification of St. Louis encephalitis virus prM and E gene coding sequences. Primer sequences were obtained from the work of Trent et al. (*Virology* 156: 293-304 (1987)). A cDNA fragment containing the genomic nucleotide region was amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR). Restriction site AfeI was engineered at the 5' terminus of the cDNA by using amplimer SLE463 (SEQ ID NO:30). An in-frame translation termination codon, followed by a NotI restriction site was introduced at the 3' terminus of the cDNA by using amplimer cSLE2447 (SEQ ID NO:31). The RT-PCR product was purified by a QIAquick™ PCR Purification Kit (Qiagen).

The double-stranded amplicon, produced by use of the two amplimers above (SEQ ID NO:30 and SEQ ID NO:31), was digested with AfeI and NotI enzymes to generate a 2004 fragment of DNA (463 to 2466nt), and inserted into the AfeI and NotI sites of a pCBJESS-M vector to form pCBSLE (nucleotide sequence, SEQ ID NO:21; amino acid sequence, SEQ ID NO:22). The pCBJESS-M was derived from the pCBamp plasmid, that contained the cytomegalovirus early gene promoter and translational control element and an engineered, modified JE signal sequence element (SEQ ID NO:27). The JE signal sequence element comprises the modified JE signal sequence at -4 (Cys to Gly) and -2 (Gly to Ser) position in the original pCBJESS plasmid.

Automated DNA sequencing using an ABI prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA) was used to confirm that the recombinant plasmid had a correct prM and E sequence as defined by Trent et al. (*Virology* 156: 293-304 (1987)).

Example 14. Preparation of recombinant plasmids containing coding sequences for yellow fever virus (YFV) prM and E proteins with JEV signal sequence. Genomic

RNA was extracted from 150 μ L of Vero cell culture medium infected with 17D-213 strain of yellow fever virus using the QIAamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA). Extracted RNA was eluted and suspended in 80 μ L of nuclease-free water, and used as a template for the amplification of yellow fever virus prM and E gene coding sequences. Primer sequences were obtained from the work of dos Santos et al. (*Virus Research* 35: 35-41 (1995)). A cDNA fragment containing the genomic nucleotide region was amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR). Restriction site AfeI was engineered at the 5' terminus of the cDNA by using amplicon YF482 (SEQ ID NO:28). An in-frame translation termination codon, followed by a NotI restriction site was introduced at the 3' terminus of the cDNA by using amplicon cYF2433 (SEQ ID NO:29). The RT-PCR product was purified by a QIAquick™ PCR Purification Kit (Qiagen).

The double-stranded amplicon, produced by use of the two amplicons above (SEQ ID NO:28 and SEQ ID NO:29), was digested with AfeI and NotI enzymes to generate a 1971 fragment of DNA (482 to 2452nt), and inserted into the AfeI and NotI sites of a pCBJESS-M vector to form pCBYF (nucleotide sequence, SEQ ID NO:23; amino acid sequence, SEQ ID NO:24). The pCBJESS-M was derived from the pCBamp plasmid, that contained the cytomegalovirus early gene promoter and translational control element and an engineered JE signal sequence element (SEQ ID NO:27). The JE signal sequence element comprises the modified JE signal sequence at -4 (Cys to Gly) and -2 (Gly to Ser) position of JESS in the pCBJESS plasmid.

Automated DNA sequencing using an ABI prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA) was used to confirm that the recombinant plasmid had a correct prM and E sequence as defined by dos Santos et al. (*Virus Research* 35: 35-41 (1995)).

Example 15. Preparation of recombinant plasmids containing coding sequences for Powassan virus prM and E antigens with JEV signal sequence. Genomic RNA was

extracted from 150 µL of Vero cell culture medium infected with LB strain of Powassan virus using the QIAamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA). Extracted RNA was eluted and suspended in 80 µl of nuclease-free water, and used as a template for the amplification of Powassan virus prM and E gene coding sequences.

- 5 Primer sequences were obtained from the work of Mandl et al. (*Virology* 194: 173-184 (1993)). A cDNA fragment containing the genomic nucleotide region was amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR). Restriction site AfeI was engineered at the 5' terminus of the cDNA by using amplicon POW454 (SEQ ID NO:25). An in-frame translation termination codon, followed by a NotI restriction site
10 was introduced at the 3' terminus of the cDNA by using amplicon cPOW2417 (SEQ ID NO:26). The RT-PCR product was purified by a QIAquick™ PCR Purification Kit (Qiagen).

- The double-stranded amplicon, produced by use of the two amplicons above (SEQ ID NO:25 and SEQ ID NO:26), was digested with AfeI and NotI enzymes to
15 generate a 1983 bp fragment of DNA (454 to 2436nt), and inserted into the AfeI and NotI sites of a pCBJESS-M vector to form pCBPOW (nucleotide sequence, SEQ ID NO:19; amino acid sequence, SEQ ID NO:20). The pCBJESS-M was derived from the pCBamp plasmid, that contained the cytomegalovirus early gene promoter and translational control element and an engineered JE signal sequence element (SEQ ID
20 NO:27). The JE signal sequence element comprises the modified JE signal sequence at -4 (Cys to Gly) and -2 (Gly to Ser) position of JESS in the pCBJESS plasmid.

- Automated DNA sequencing using an ABI prism 377 Sequencer (Applied Biosystems/Perkin Elmer, Foster City, CA) was used to confirm that the recombinant plasmid had a correct prM and E sequence as defined by Mandl et al. (*Virology*
25 194:173-184, (1993)).

Example 16. Preparation of plasmids containing coding sequences for dengue serotype 2 structural proteins. Procedures such as those carried out for other

flaviviruses (see Examples 1, 9 and 12-15) are to be followed to prepare vectors including nucleic acid TU's for dengue serotype 2 antigens. According to the examples, the amplimers used for construction of the vectors may be chosen to engineer the normal dengue virus signal sequence or they may be chosen so as to engineer a
5 signal sequence from another flavivirus, such as a modified Japanese encephalitis virus signal sequence.

A plasmid containing the dengue serotype 2 gene region from prM to E is to be constructed. The dengue serotype 2 prM and E genes (Deubel et al., *Virology* 155:365-377 (1986); Gruenberg et al., *J. Gen. Virol.* 69: 1301-1398 (1988); Hahn et al.,
10 *Virology* 162:167-180 (1988)) are to be ligated into a plasmid such as pCDNA3, and then excised and cloned into vectors such as pCBamp, pCEP4, pREP4, or pRc/RSV (supplied by Invitrogen, Carlsbad, CA) to enable expression. If necessary, a dengue serotype 2 virus-specific sequence encoded in a cDNA sequence may be amplified using a procedure such as the polymerase chain reaction (PCR). Alternatively, if the
15 viral RNA is the source of the gene region, a DNA sequence may be amplified by a RT-PCR procedure. A DNA fragment including an initiation codon at the 5' end, and a termination codon at the 3' end is to be cloned into an expression vector at an appropriate restriction nuclease-specific site, in such a way that the cytomegalovirus (CMV) immediate early (IE) promoter, an initiation codon, and a terminator, are
20 operably linked to the dengue serotype 2 virus sequence.

Example 17. Vaccination of mice using a dengue serotype 2 DNA vaccine. The dengue serotype 2 nucleic TU vaccine encoding the gene region from prM to E prepared in Example 16 is to be suspended in a suitable pharmaceutical carrier, such as water for injection or buffered physiological saline, and injected intramuscularly into
25 groups of weanling mice. Control groups receive a comparable plasmid preparation lacking the dengue serotype 2 specific genes. The generation of dengue serotype 2-specific antibodies, and/or of dengue serotype 2-specific immune system cytotoxic cells, is to be assessed at fixed intervals thereafter, for example at weekly intervals. At

about two to four months after administration of the nucleic acid TU vaccine, mice are to be challenged with dengue serotype 2 virus. Levels of viremia are to be assessed at appropriate intervals thereafter, such as every second day. Passive protection by maternal antibody is to be assessed as indicated in Example 8.

14114.0332U3

Table 1. Transient expression of JE prM and E proteins by various recombinant plasmids in two transferred cell lines.

	Vector				Recombinant Plasmid	IFA intensity/percentage of antigen-positive cells*	
	Promotor	Intron	Poly(A)	ORI		COS-1	COS-7
pCDNA3	CMV	No	BGH	SV40	pCDJE2-7	3+/40	3+/35
pCBamp	CMV	No	BGH	No	pCBJE1-14	3+/45	nd
pCIBamp	CMV	Yes	BGH	No	pCIBJES14	3+/39	nd
pCEP4	CMV	No	SV40	Orig	pCEJE	2+/4	2+/3
pREP4	RSV	No	SV40	Orig	pREJE	1+/3	1+/2
pRe/RSV	RSV	No	BGH	SV40	pRCJE	1+/3	1+/3
pCDNA3	CMV	No	BGH	SV40	pCDNA3/CAT	-	-

*Various cell lines were transformed with pCDNA3/CAT (negative control), pCDJE2-7, pCBJE1-14, pCIBJES14, pCEJEm pREJE, or pRCJE. Cells are trypsinized 48 hours later and tested by an indirect immunofluorescent antibody assay (IFA) with JE virus-specific HIAF. Data are presented as the intensity (scale of 1+ to 4+) and the percentage of IFA positive cells. The pCDNA3/CAT transformed cells were used as the negative control.

Table 2. Characterization of proteins expressed by a pCDJE2-7 stably transformed clone (JE-4B) of COS-1 cells with JE virus-reactive antibodies.

Mab or antiserum	Biological Activity of Mab		Immunofluorescent intensity of cells	
	Specificity	Biological Function	JEV infected	4B
Mab:				
MC3	JEV Specific		2+	2+
2F2	JEV Specific	HI, N	4+	4+
112	JEV Specific		4+	4+
503	JEV Specific	N	4+	3+
109	Subgroup	HI	2+	1+
N.04	Subgroup	HI, N	3+	4+
201	Subgroup		1+	1+
203	Subgroup		4+	3+
204	Subgroup		2+	2+
301	Subgroup	HI	2+	2+
504	Flavivirus		4+	4+
6B6C-1	Flavivirus		2+	2+
3B4C-4	VEE		-	-
H1AF:				
Anti-JEV			4+	3+
Anti-WEE			-	-
PBS			-	-

1092511.0404
F0404-512550

Table 3. Persistence of the immune response in mice immunized with pCDJE2-7 or JE-VEX vaccine.

	ELISA Titer (log ₁₀)						PRNT _{50%} Titer		
	3 wks	6 wks	9 wks	23 wks	40 wks	60 wks*	3 wks	6 wks	9 wks
1x pCDJE2-7	2.6-3.2	3.8-5.0	3.8-4.4	>3.2	>3.2	2.4, 2.4, 3.8, 4.4	<20	20	40-160
2x pCDJE2-7	2.6-3.8	4.4	3.8-4.4	>3.2	>3.2	2.6, 3.8, 3.8	<20	20-40	40-160
2x JE-VAX	2.6-3.8	4.4-5.0	3.8-5.6	>3.2	>3.2	<2, <2, 4.4	<20	20-40	20-160
2x pCDNA3/CAT	<2	<2	<2	ND	ND	<2	<20	<20	<20

Mice were inoculated with 1 or 2, 100 µg/dose plasmid DNA, or 1/5 human dose of JE-VAX vaccine. Sera were collected for testing prior to the second immunization.

* Individual serum titers.

Table 4. The age-dependent percent seropositive rate in mice following vaccination with various JEV vaccines.

	3-day old		3-week old	
	3 weeks PV	7 weeks PV	3 weeks PV	7 weeks PV
JE-VAX	0	0	100	100
pCDNA3/CAT	0	0	0	0
pCDJE2-7	40	60	90	90
pC1BJES14	10	60	80	100
pCBE1-14	80	100	100	100

2025-09-10 10:50:50

Table 5. Protection from JEV challenge in 8 week old mice following vaccination at 3 days old with various JEV vaccines.

Vaccine	Pre-challenge JEV seroconversion	Days post-challenge survival rate (%)				
		6	7	8	9	21
JE-VAX	0	100	100	60	40	40
pCDNA3/CAT	0	100	80	30	30	30
pCDJE2-7	60	100	100	100	100	100
pC1BJES14	60	100	100	100	100	100
pCBE1-14	100	100	100	100	100	100

092615-040401

Table 6. Evaluation of the ability of maternal antibody from JEV-nucleic acid-vaccinated female mice to protect their pups from fatal JEV encephalitis.

Vaccinated mother		JEV challenged pups		
Vaccine	PRNT _{90%}	Challenge age (days)	No. survival ¹	ELISA ²
1 x pCDJE2-7	40	4	0/11	
2 x pCDJE2-7	80	4	12/12	12/12
2 x JE-VAX	20	3	0/16	
2 x pCDNA-3/CAT	<10	5	0/14	
1 x pCDJE2-7	20	15	5/11	5/5
2 x pCDJE2-7	40	14	8/12	7/8
2 x JE-VAX	80	13	5/5	5/5
2 x pCDNA-3/CAT	<10	14	0/14	

Mice were inoculated intramuscularly with 1 or 2, 100 µg dose of plasmid DNA, or subcutaneously with two, 1/5 human dose of JE-VAX vaccine. Sera were collected 9 weeks post-vaccination for PRNT testing prior to mating with non-immune male.

¹: No Survivors/total for each litter.

²: Number of JEV ELISA-antibody-positive animals (titer ≥ 1:400)/No. of survivors; sera were collected for testing 12 weeks after challenge.

092615-04001
T04040-5T9260

1. An isolated nucleic acid comprising a transcriptional unit encoding a signal sequence of a structural protein of a first flavivirus and an immunogenic flavivirus antigen of a second flavivirus, wherein the transcriptional unit directs the synthesis of the antigen.

3. The nucleic acid of claim 1, wherein the immunogenic flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.

5. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of yellow fever virus.

7. The nucleic acid of claim 1, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus and an M protein and an E protein of Powassan virus.

8. The nucleic acid of claim 1, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.
9. The nucleic acid of claim 8, wherein the antigen is both the M protein and the E protein of a flavivirus.
10. The nucleic acid of claim 1, wherein the nucleic acid is DNA.
11. The nucleic acid of claim 10, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.
12. The nucleic acid of claim 1, wherein the transcriptional unit comprises a control sequence disposed appropriately such that it operably controls the synthesis of the antigen.
13. The nucleic acid of claim 12, wherein the control sequence is the cytomegalovirus immediate early promoter.
14. The nucleic acid of claim 1, comprising a Kozak consensus sequence located at a translational start site for a polypeptide comprising the antigen encoded by the TU.
15. The nucleic acid of claim 1 wherein the transcriptional unit comprises a poly-A terminator.
16. A cell comprising the nucleic acid of claim 1.

104040-51152860

17. A composition comprising the nucleic acid of claim 1 and a pharmaceutically acceptable carrier.
18. A method of immunizing a subject against infection by a flavivirus, comprising administering to the subject an effective amount of the composition of claim 17.
19. The method of claim 18, wherein the flavivirus antigen is of a flavivirus selected from the group consisting of yellow fever virus, dengue serotype 1 virus, dengue serotype 2 virus, dengue serotype 3 virus, dengue serotype 4 virus, Japanese encephalitis virus, Powassan virus and West Nile virus.
20. The method of claim 18, wherein the antigen is selected from the group consisting of an M protein of a flavivirus, an E protein of a flavivirus, both an M protein and an E protein of a flavivirus, a portion of an M protein of a flavivirus, a portion of an E protein of a flavivirus and both a portion of an M protein of a flavivirus and a portion of an E protein of a flavivirus or any combination thereof.
21. The method of claim 20, wherein the antigen is both the M protein and the E protein of a flavivirus, and wherein a cell within the body of the subject, after incorporating the nucleic acid within it, secretes subviral particles comprising the M protein and the E protein.
22. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of West Nile virus.
23. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of yellow fever virus.

24. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of St. Louis encephalitis virus.
25. The method of claim 18, wherein the transcriptional unit encodes a signal sequence of Japanese encephalitis virus, and an M protein and an E protein of Powassan virus.
26. The method of claim 18, comprising administering the composition to the subject in a single dose.
27. The method of claim 18, wherein the composition is administered via a parenteral route.
28. The nucleic acid of claim 1, wherein the antigen is a St. Louis encephalitis virus antigen.
29. The method of claim 18, wherein the antigen is a St. Louis encephalitis virus antigen.
30. The nucleic acid of claim 1, wherein the antigen is a Japanese encephalitis virus antigen.
31. The method of claim 18, wherein the antigen is a Japanese encephalitis virus antigen.
32. The nucleic acid of claim 1, wherein the antigen is a yellow fever virus antigen.
33. The method of claim 18, wherein the antigen is a yellow fever virus antigen.

092615-04040
FOH040-54T92660

34. The nucleic acid of claim 1, wherein the antigen is a dengue virus antigen.
35. The method of claim 18, wherein the antigen is a dengue virus antigen.
36. The nucleic acid of claim 1, wherein the antigen is a West Nile virus antigen.
37. The method of claim 18, wherein the antigen is a West Nile virus antigen.
38. An antigen produced from the nucleic acid of claim 1.
39. A method of detecting a flavivirus antibody in a sample, comprising:
 - (a) contacting the sample with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and
 - (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antibody in the sample.
40. An antibody produced in response to immunization by the antigen of claim 38.
41. A method of detecting a flavivirus antigen in a sample, comprising:
 - (a) contacting the sample with the antibody of claim 40 under conditions whereby an antigen/antibody complex can form; and
 - (b) detecting antigen/antibody complex formation, thereby detecting a flavivirus antigen in a sample.
42. A method of diagnosing a flavivirus infection in a subject, comprising:
 - (a) contacting a sample from the subject with the antigen of claim 38 under conditions whereby an antigen/antibody complex can form; and
 - (b) detecting antigen/antibody complex formation, thereby diagnosing a flavivirus infection in a subject.

Abstract

The present invention encompasses isolated nucleic acids containing transcriptional units which encode a signal sequence of one flavivirus and an immunogenic flavivirus antigen of a second flavivirus. The invention further
5 encompasses a nucleic acid and protein vaccine and the use of the vaccine to immunize a subject against flavivirus infection. The invention also provides antigens encoded by nucleic acids of the invention, antibodies elicited in response to the antigens and use of the antigens and/or antibodies in detecting flavivirus or diagnosing flavivirus infection.

092615-040401
FOI010-S152260

DECLARATION FOR PATENT APPLICATION

☒ Original ☐ Supplemental ☐ Substitute ☐ PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**NUCLEIC ACID VACCINES FOR PREVENTION OF FLAVIVIRUS INFECTION,**" which is described and claimed in the specification

☒ (check one) ☒ which is attached hereto, or
☐ which was filed on , as Application Serial No. and with amendments through
☐ (if applicable), or
☐ in International Application No. PCT/, filed , and as amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

1 I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any
2 foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application
3 which designated at least one country other than the United States of America, listed below and have also
4 identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any
5 PCT international application having a filing date before that of the application on which priority is
6 claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
PCT/US99/12298	PCT	3 June 1999	X	

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE
60/087,908	4 June 1998

ATTORNEY DOCKET NO. 14114.0332U3
PAGE 2 OF 2

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED
09/701,536	11/29/00		X	

09/04/01 09:00:00
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: GWONG-JEN J. CHANG

Inventor's signature:

Gwong-Jen Chang

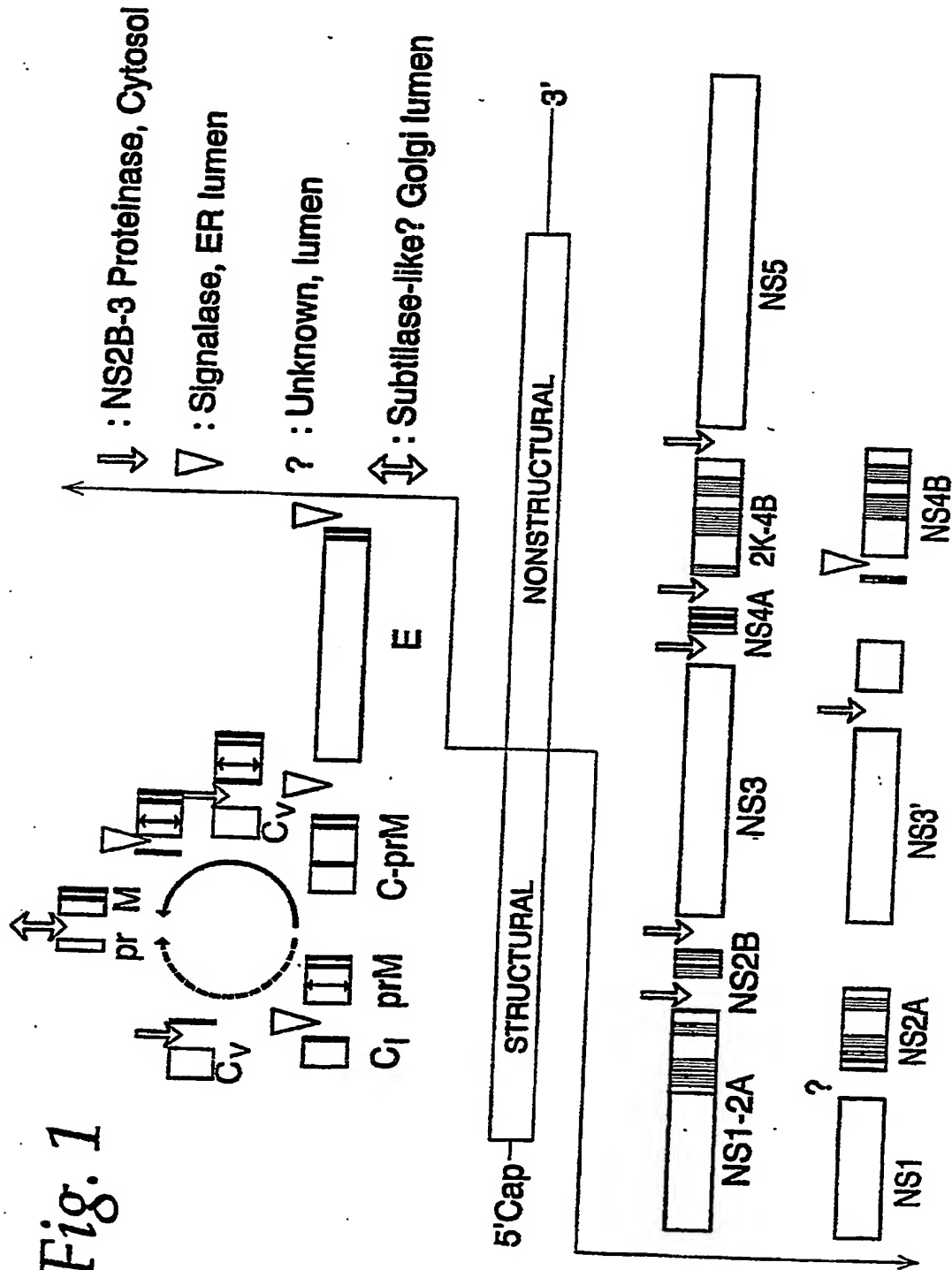
Date: April 2, 2001

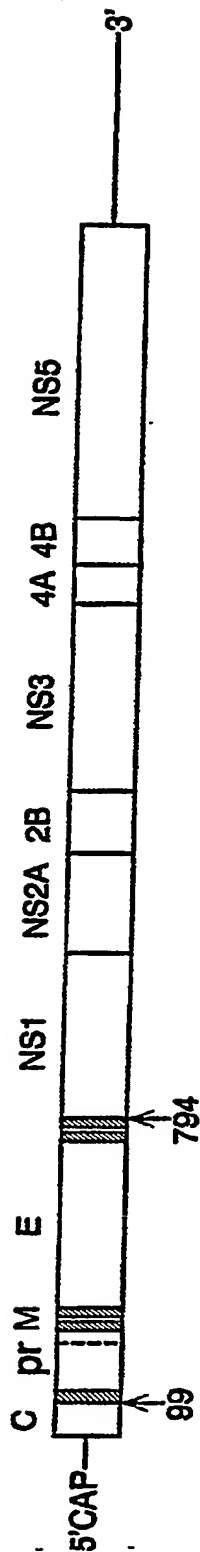
Residence: 4237 Beaver Creek Drive, Fort Collins, CO 80526

Post Office Address: 4237 Beaver Creek Drive, Fort Collins, CO 80526

Citizenship: USA

Fig. 1





14DV389: 5' CTT GGTACC TCTAGA GCGCGGCC ATG GGC AGA AAG CAA AAC AAA AGA

KpnI XbaI Kozak seq M G R K Q N K R

99 ↓

794 ↓

F L A T N V H A #

TTC TTA GCG ACCAAT GTG CAT GCT TAA

NotI

c14DV2453: AAG AAT CGC TGG TTA CAC GTA CGA ATT CAAACT CGCGGCGG TTTTCITTT 5'

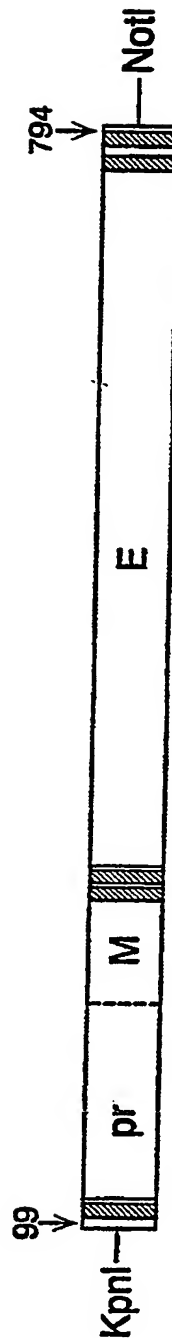


Fig. 2

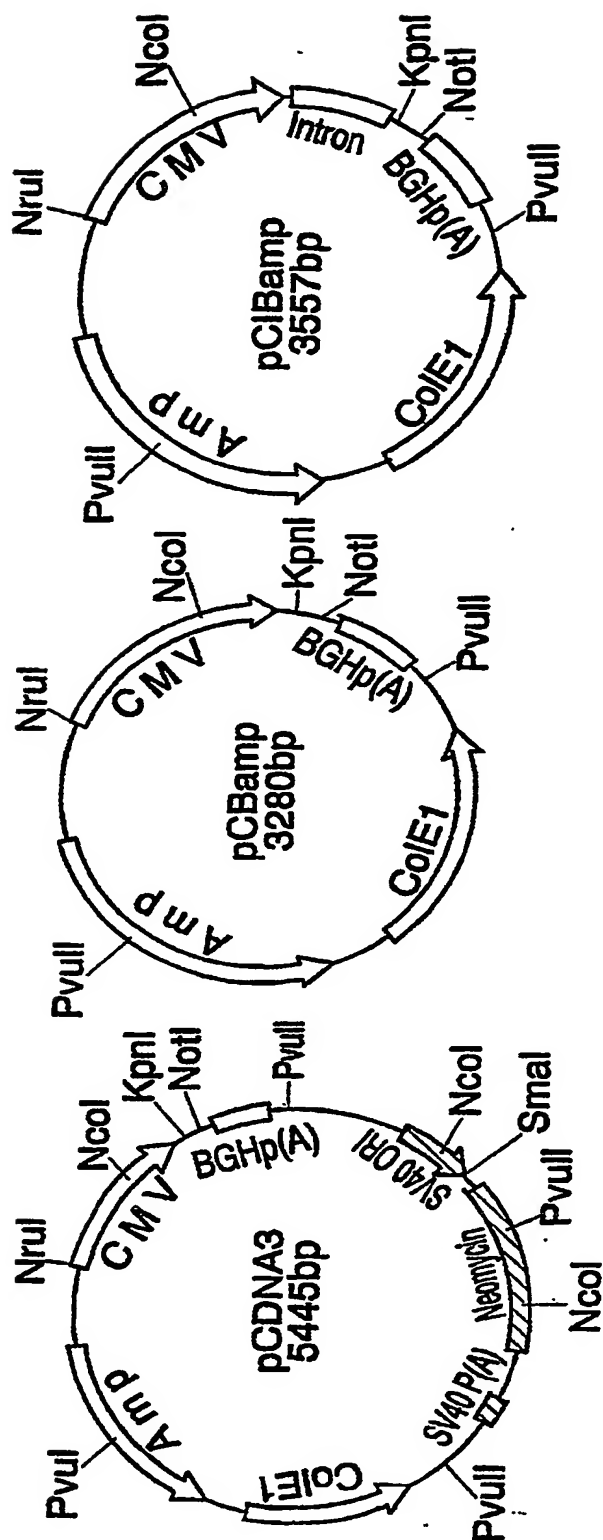


Fig. 3

09826115.040401

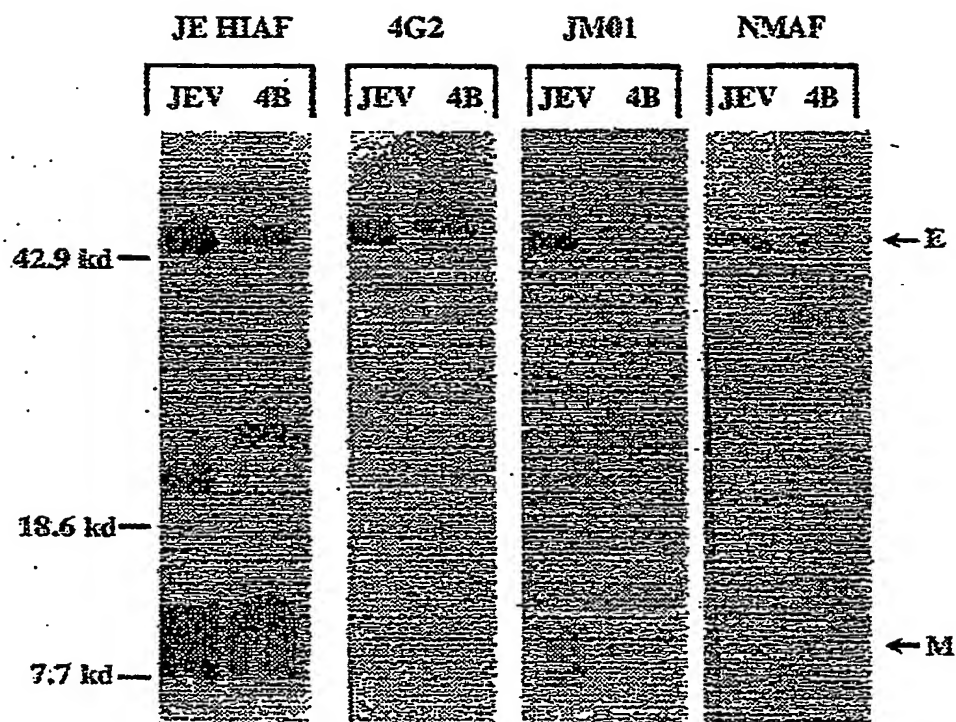


Fig. 4

TDH0-STF3280

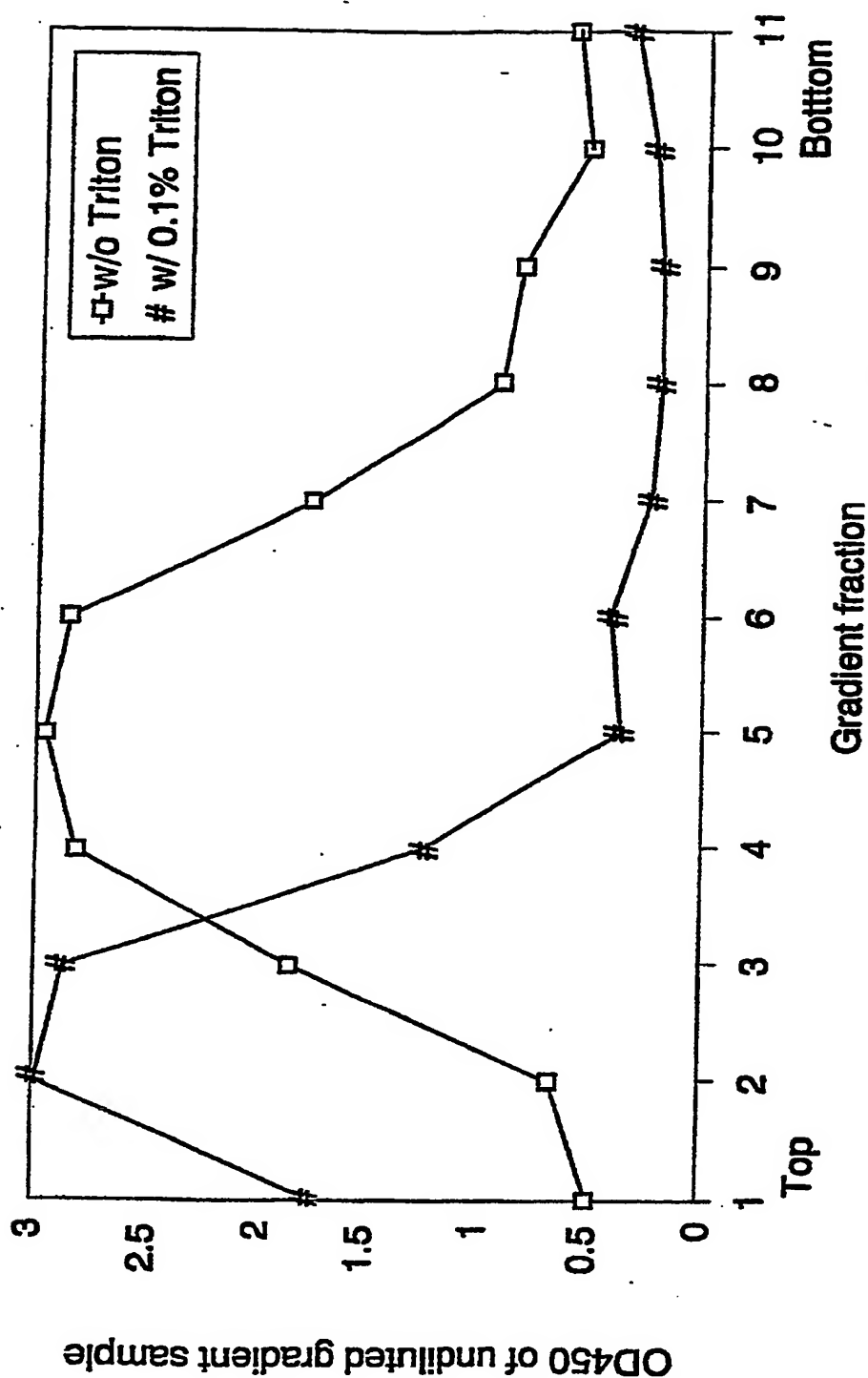


Fig. 5

SEQUENCE LISTING

<110> Chang, Gwong-Jen J

<120> Nucleic Acid Vaccines for Prevention of
Flavivirus Infection

<130> 14114.0332U3

<150> PCT/US99/12298

<151> 1999-06-03

<150> 09/701,536

<151> 2000-11-29

<150> 60/087,908

<151> 1998-06-04

<160> 31

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
synthetic construct

<221> CDS

<222> (25)...(48)

<221> misc_feature

<222> 1-48

<223> Amplimer 14DV389

<400> 1

cttgggtacct cttagagccgc cgcc atg ggc aga aag caa aac aaa aga
Met Gly Arg Lys Gln Asn Lys Arg

48

1

5

<210> 2

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
synthetic construct

104040-5T93360

```
<221> misc_feature
<222> 1-50
<223> Amplimer c14DV2453
```

```
<400> 3
ttttcttttg cggccgctca aacttaagca tgcacattgg tcgctaagaa
```

50

```
<210> 4
<211> 48
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of artificial sequence; note =
        synthetic construct
```

```
<221> CDS
<222> (25)...(48)
```

```
<221> misc_feature
<222> (1)...(48)
<223> Amplimer YFDV389
```

```
<400> 4
cttggtacct ctagagccgc cgcc atg cgt tcc cat gat gtt ctg act
      Met Arg Ser His Asp Val Leu Thr
      1               5
```

48

```
<210> 5
<211> 8
<212> PRT
<213> Artificial Sequence
```

```
<220>
<223> Description of artificial sequence; note =
        synthetic construct
```

<220>
<223> Description of artificial sequence; note =
synthetic construct

```
<210> 9
<211> 38
<212> DNA
<213> Artificial Sequence
```

<220>
<223> Description of artificial sequence; note =
synthetic construct

```
<221> misc_feature
<222> 1-38
<223> Amplimer cSLEDV2449
```

<400> 9
ttttcttttg cggcgcctta ggcttgcaag ctggttgc

38

```
<210> 10
<211> 7500
<212> DNA
<213> Artificial Sequence
```

<220>
<223> Description of artificial sequence; note =
synthetic construct

```
<221> CDS
<222> (916) ... (3009)
```

```
<221> misc_feature
<222> 1-7500
<223> pCDJE 2-7
```

<400> 10						
gacggatcgg	gagatctccc	gatccctat	ggtcgactct	cagtacaatc	tgctctgatg	60
ccgcatagtt	aagccagtat	ctgctccctg	cttgtgtgtt	ggaggtcgct	gagtagtgcg	120
cgagcaaaat	ttaagctaca	acaaggcaag	gcttgaccga	caattgcatg	aagaatctgc	180
ttaggggttag	gcgtttttcg	ctgcttcgcg	atgtacgggc	cagatatacg	cgttgacatt	240
gattattgac	tagttattaa	tagtaaatcaa	ttacgggggc	attagttcat	agcccatata	300
tggagttccg	cgttacataa	cttacggtaa	atggcccgcc	tggctgaccg	cccaacgacc	360
cccgcccat	gacgtcaata	atgacgtatg	ttcccatagt	aacgccaata	gggaatttcc	420
attgacgtca	atgggtggac	tattttacggt	aaactgccca	cttggcagta	catcaattgt	480
atcatatgcc	aagtacgccc	cctattgacg	tcaatgacgg	taaattggccc	gcctggcatt	540
atgcccagta	catgacctta	tgggaccttc	ctacttgcca	gtacatctac	gtattagtca	600
tgcctattac	catgggtgat	cgggttttggc	agtacatcaa	tggggcggtga	tagcggtttg	660
actcaacggg	atttccaagt	ctccacccca	ttgacgtcaa	tgggagtttg	ttttggcacc	720
aaaatcaacg	ggactttcca	aaatgtctga	acaactccgc	cccattgacg	caaatggggc	780
gtaggcgtgt	acggtggggag	gtctatataa	gcagagctct	ctggctaact	agagaaccca	840
ctgcttactg	gcttatcgaa	attaatacga	ctcactatag	ggagacccaa	gcttggtacc	900

aat	cgt	gac	ttc	ata	gaa	gga	gcc	agt	gga	gcc	act	tgg	gtg	gac	ttg	1575
Asn	Arg	Asp	Phe	Ile	Glu	Gly	Ala	Ser	Gly	Ala	Thr	Trp	Val	Asp	Leu	
205					210					215					220	
gtg	ctg	gaa	gga	gat	agc	tgc	ttg	aca	atc	atg	gca	aac	gac	aaa	cca	1623
Val	Leu	Glu	Gly	Asp	Ser	Cys	Leu	Thr	Ile	Met	Ala	Asn	Asp	Lys	Pro	
				225					230					235		
aca	ttg	gac	gtc	cgc	atg	att	aac	atc	gaa	gct	agc	caa	ctt	gct	gag	1671
Thr	Leu	Asp	Val	Arg	Met	Ile	Asn	Ile	Glu	Ala	Ser	Gln	Leu	Ala	Glu	
			240					245					250			
gtc	aga	agt	tac	tgc	tat	cat	gct	tca	gtc	act	gac	atc	tcg	acg	gtg	1719
Val	Arg	Ser	Tyr	Cys	Tyr	His	Ala	Ser	Val	Thr	Asp	Ile	Ser	Thr	Val	
		255					260					265				
gct	cgg	tgc	ccc	acg	act	gga	gaa	gcc	cac	aac	gag	aag	cga	gct	gat	1767
Ala	Arg	Cys	Pro	Thr	Thr	Gly	Glu	Ala	His	Asn	Glu	Lys	Arg	Ala	Asp	
	270					275						280				
agt	agc	tat	gtg	tgc	aaa	caa	ggc	ttc	act	gac	cgt	ggg	tgg	ggc	aac	1815
Ser	Ser	Tyr	Val	Cys	Lys	Gln	Gly	Phe	Thr	Asp	Arg	Gly	Trp	Gly	Asn	
285					290					295					300	
gga	tgt	gga	ctt	ttc	ggg	aag	gga	agc	att	gac	aca	tgt	gca	aaa	ttc	1863
Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser	Ile	Asp	Thr	Cys	Ala	Lys	Phe	
				305					310					315		
tcc	tgc	acc	agt	aaa	gcg	att	ggg	aga	aca	atc	cag	cca	gaa	aac	atc	1911
Ser	Cys	Thr	Ser	Lys	Ala	Ile	Gly	Arg	Thr	Ile	Gln	Pro	Glu	Asn	Ile	
			320					325					330			
aaa	tac	gaa	gtt	ggc	att	ttt	gtg	cat	gga	acc	acc	act	tcg	gaa	aac	1959
Lys	Tyr	Glu	Val	Gly	Ile	Phe	Val	His	Gly	Thr	Thr	Thr	Ser	Glu	Asn	
		335					340					345				
cat	ggg	aat	tat	tca	gcg	caa	gtt	ggg	gcg	tcc	cag	gcg	gca	aag	ttt	2007
His	Gly	Asn	Tyr	Ser	Ala	Gln	Val	Gly	Ala	Ser	Gln	Ala	Ala	Lys	Phe	
	350					355					360					
aca	gta	aca	ccc	aat	gct	cct	tcg	ata	acc	ctc	aaa	ctt	ggt	gac	tac	2055
Thr	Val	Thr	Pro	Asn	Ala	Pro	Ser	Ile	Thr	Leu	Lys	Leu	Gly	Asp	Tyr	
365					370					375					380	
gga	gaa	gtc	aca	ctg	gac	tgt	gag	cca	agg	agt	gga	ctg	aac	act	gaa	2103
Gly	Glu	Val	Thr	Leu	Asp	Cys	Glu	Pro	Arg	Ser	Gly	Leu	Asn	Thr	Glu	
				385					390					395		
gcg	ttt	tac	gtc	atg	acc	gtg	ggg	tca	aag	tca	ttt	ctg	gtc	cat	agg	2151
Ala	Phe	Tyr	Val	Met	Thr	Val	Gly	Ser	Lys	Ser	Phe	Leu	Val	His	Arg	
			400					405								

THE **WORLD'S** **LARGEST** **BOOK** **STORE**

gcc gtt cac caa gtg ttt ggt ggt gcc ttc aga aca ctc ttt ggg gga 2871
 Ala Val His Gln Val Phe Gly Gly Ala Phe Arg Thr Leu Phe Gly Gly
 640 645 650

atg tct tgg atc aca caa ggg cta atg ggt gcc cta ctg ctc tgg atg 2919
 Met Ser Trp Ile Thr Gln Gly Leu Met Gly Ala Leu Leu Leu Trp Met
 655 660 665

ggc gtc aac gca cga gac cga tca att gct ttg gcc ttc tta gcc aca 2967
 Gly Val Asn Ala Arg Asp Arg Ser Ile Ala Leu Ala Phe Leu Ala Thr
 670 675 680

ggg ggt gtg ctc gtg ttc tta gcg acc aat gtg cat gct taa 3009
 Gly Gly Val Leu Val Phe Leu Ala Thr Asn Val His Ala *
 685 690 695

ttagtgtgag cggcgcgtcg agcatgcac tagagggccc tattctatag tgtcacctaa 3069
 atgctagagc tcgctgatca gcctcgactg tgccttctag ttgccagcca tctgtgtgtt 3129
 gcccctcccc cgtgccttcc ttgacctgg aaggtgccac tcccactgtc ctttcctaat 3189
 aaaatgagga aattgcatcg cattgtctga gtagggtgca ttctattctg ggggggtgggg 3249
 tggggcagga cagcaagggg gaggattggg aagacaatag caggcatgct ggggatgcgg 3309
 tgggctctat ggcttctgag gcggaagaa ccagctgggg ctctaggggg tatccccacg 3369
 cgccctgtag cggcgcatta agcgcggcgg gtgtgggtgg tactcgcagc gtgaccgcta 3429
 cactgcgag cgccctagcg ccgcctcctt tcgcttctt cccctccttt ctcgccacgt 3489
 tcgcccgtt tcccgcgtcaa gctctaaatc ggggcacccc tttagggttc cgatttagtg 3549
 ctttacggca cctcgacccc aaaaaacttg attagggtga tggttcacgt agtgggcat 3609
 cgccctgata gacgggtttt cgccctttga cgttggagtc cacgttcttt aatagtggac 3669
 tcttgttcca aactggaaca acactcaacc ctatctcggt ctattctttt gatattataag 3729
 ggattttggg gatttcggcc tatttggttaa aaaatgagct gatttaacaa aaatttaacg 3789
 cgaattaatt ctgtggaatg tgtgtcagtc aggggtgtgga aagtccccag gctccccagg 3849
 caggcagaag tatgcaaaag atgcatacga attagtcagc aaccagggtg ggaaagtccc 3909
 caggctcccc agcaggcaga agtatgcaaa gcatgcatct caattagtca gcaaccatag 3969
 tcccgcctt aactccgccc atcccgcctt taactccgct cagttccgct cattctccgc 4029
 cccatggctg actaattttt tttatttatg cagaggccga ggccgcctct gcctctgagc 4089
 tattccagaa gtatgagga ggcttttttg gaggcctagg cttttgcaaa aagctcccg 4149
 gagcttgat atccattttc ggatctgatc aagagacagg atgaggatcg ttctcgcatga 4209
 ttgaacaaga tggattgcac gcaggttctc cggcgcgttg ggtggagagg ctattcggtc 4269
 atgactgggc acaacagaca atcggctgct ctgatgcgc cgtgttccgg ctgtcagcgc 4329
 aggggcgccc ggttcttttt gtcaagaccg acctgtccgg tgccctgaat gaactgcagg 4389
 acgaggcagc gcggctatcg tggctggcca cgacgggctg tcttgcgca gctgtgctcg 4449
 acgttgtcac tgaagcggga agggactggc tgctattggg cgaagtgcg gggcaggatc 4509
 tctgtcatc tcaccttgct cctgccgaga aagtatccat catggctgat gcaatgcggc 4569
 ggctgcatac gcttgatccg gctacctgcc cattcgacca ccaagcgaat catcgcatcg 4629
 agcagacagc tactcggatg gaagccggtc ttgtcgatca ggatgatctg gacgaagagc 4689
 atcaggggct cgcgccagcc gaactgttcg ccaggctcaa ggccgcgatg cccgacggcg 4749
 aggatctcgt cgtgacccat ggcatgcct gcttgccgaa tatcatggtg gaaaatggcc 4809
 gcttttcttg attcatcgac tgtggccggc tgggtgtggc ggaccgctat caggacatag 4869
 cgttggctac cgtgatatt gctgaagagc ttggcggcga atgggctgac cgcttctctg 4929
 tgctttacgg tatcgccgct cccgattcgc agcgcacgc cttctatcgc cttcttgacg 4989
 agttctcttg agcgggaactc tggggttcga aatgaccgac caagcgacgc ccaacctgcc 5049
 atcacagat ttcgattcca ccgccgcct ctatgaaagg ttgggcttcg gaatcgtttt 5109
 ccgggagcc ggctggatga tctccagcg cggggatctc atgctggagt tcttcgcca 5169
 ccccaacttg tttattgcag cttataatgg ttacaaataa agcaatagca tcacaaattt 5229

000001: 5775260

THE UNIVERSITY OF CHICAGO

<223> pCDJE 2-7

<400> 11

Met Gly Arg Lys Gln Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile Met
 1 5 10 15
 Trp Leu Ala Ser Leu Ala Val Val Ile Ala Cys Ala Gly Ala Met Lys
 20 25 30
 Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile Asn Asn Thr Asp
 35 40 45
 Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly Glu Asn Arg Cys
 50 55 60
 Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu Asp Thr Ile Thr
 65 70 75 80
 Tyr Glu Cys Pro Lys Leu Thr Met Gly Asn Asp Pro Glu Asp Val Asp
 85 90 95
 Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys Thr
 100 105 110
 Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val Ser Val Gln Thr
 115 120 125
 His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala Trp Leu Asp Ser
 130 135 140
 Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile Arg
 145 150 155 160
 Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly Trp Met Leu Gly
 165 170 175
 Ser Asn Asn Gly Gln Arg Val Val Phe Thr Ile Leu Leu Leu Val
 180 185 190
 Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly Asn Arg Asp Phe
 195 200 205
 Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly
 210 215 220
 Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro Thr Leu Asp Val
 225 230 235 240
 Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu Val Arg Ser Tyr
 245 250 255
 Cys Tyr His Ala Ser Val Thr Asp Ile Ser Thr Val Ala Arg Cys Pro
 260 265 270
 Thr Thr Gly Glu Ala His Asn Glu Lys Arg Ala Asp Ser Ser Tyr Val
 275 280 285
 Cys Lys Gln Gly Phe Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu
 290 295 300
 Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys Phe Ser Cys Thr Ser
 305 310 315 320
 Lys Ala Ile Gly Arg Thr Ile Gln Pro Glu Asn Ile Lys Tyr Glu Val
 325 330 335
 Gly Ile Phe Val His Gly Thr Thr Thr Ser Glu Asn His Gly Asn Tyr
 340 345 350
 Ser Ala Gln Val Gly Ala Ser Gln Ala Ala Lys Phe Thr Val Thr Pro
 355 360 365
 Asn Ala Pro Ser Ile Thr Leu Lys Leu Gly Asp Tyr Gly Glu Val Thr
 370 375 380
 Leu Asp Cys Glu Pro Arg Ser Gly Leu Asn Thr Glu Ala Phe Tyr Val
 385 390 395 400
 Met Thr Val Gly Ser Lys Ser Phe Leu Val His Arg Glu Trp Phe His
 405 410 415

F00000-5T52860

[illegible]

cttggtaccc gtctcggcgc cgtgaccctc tcgaacttcc agggca

43

<400> 15

gacggatcgg	gagatctccc	gatcccctat	ggtgcactct	cagtacaatc	tgctctgatg	60
ccgcatagtt	aagccagtat	ctgctccctg	cttgtgtgtt	ggaggtcgct	gagtagtgcg	120
cgagcaaaat	ttaagctaca	acaaggcaag	gcttgaccga	caattgcatt	aaqaatctgc	180

Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala
1 5 10

aca agg tat ttg gta aaa aca gaa tca tgg atc ttg agg aac cct gga 1381
Thr Arg Tyr Leu Val Lys Thr Glu Ser Trp Ile Leu Arg Asn Pro Gly
145 150 155

THE **NEW** **AMERICAN** **DICTIONARY** **OF** **THE** **ENGLISH** **LANGUAGE**

tat gcc ctg gtg gca gcc gtc att ggt tgg atg ctt ggg agc aac acc 1429
 Tyr Ala Leu Val Ala Ala Val Ile Gly Trp Met Leu Gly Ser Asn Thr
 160 165 170

atg cag aga gtt gtg ttt gtc gtg cta ttg ctt ttg gtg gcc cca gct 1477
 Met Gln Arg Val Val Phe Val Val Leu Leu Leu Leu Val Ala Pro Ala
 175 180 185

tac agc ttc aac tgc ctt gga atg agc aac aga gac ttc ttg gaa gga 1525
 Tyr Ser Phe Asn Cys Leu Gly Met Ser Asn Arg Asp Phe Leu Glu Gly
 190 195 200 205

gtg tct gga gca aca tgg gtg gat ttg gtt ctc gaa ggc gac agc tgc 1573
 Val Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly Asp Ser Cys
 210 215 220

gtg act atc atg tct aag gac aag cct acc atc gat gtg aag atg atg 1621
 Val Thr Ile Met Ser Lys Asp Lys Pro Thr Ile Asp Val Lys Met Met
 225 230 235

aat atg gag gcg gcc aac ctg gca gag gtc cgc agt tat tgc tat ttg 1669
 Asn Met Glu Ala Ala Asn Leu Ala Glu Val Arg Ser Tyr Cys Tyr Leu
 240 245 250

gct acc gtc agc gat ctc tcc acc aaa gct gcg tgc ccg acc atg gga 1717
 Ala Thr Val Ser Asp Leu Ser Thr Lys Ala Ala Cys Pro Thr Met Gly
 255 260 265

gaa gct cac aat gac aaa cgt gct gac cca gct ttt gtg tgc aga caa 1765
 Glu Ala His Asn Asp Lys Arg Ala Asp Pro Ala Phe Val Cys Arg Gln
 270 275 280 285

gga gtg gtg gac agg ggc tgg ggc aac ggc tgc gga cta ttt ggc aaa 1813
 Gly Val Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys
 290 295 300

gga agc att gac aca tgc gcc aaa ttt gcc tgc tct acc aag gca ata 1861
 Gly Ser Ile Asp Thr Cys Ala Lys Phe Ala Cys Ser Thr Lys Ala Ile
 305 310 315

gga aga acc atc ttg aaa gag aat atc aag tac gaa gtg gcc att ttt 1909
 Gly Arg Thr Ile Leu Lys Glu Asn Ile Lys Tyr Glu Val Ala Ile Phe
 320 325 330

gtc cat gga cca act act gtg gag tgc cac gga aac tac tcc aca cag 1957
 Val His Gly Pro Thr Thr Val Glu Ser His Gly Asn Tyr Ser Thr Gln
 335 340 345

gtt gga gcc act cag gca ggg aga ttc agc atc act cct gcg gcg cct 2005
 Val Gly Ala Thr Gln Ala Gly Arg Phe Ser Ile Thr Pro Ala Ala Pro
 350 355 360 365

tca tac aca cta aag ctt gga gaa tat gga gag gtg aca gtg gac tgt 2053

000000-04001

Ser Tyr Thr Leu Lys Leu Gly Glu Tyr Gly Glu Val Thr Val Asp Cys
 370 375 380

gaa cca cgg tca ggg att gac acc aat gca tac tac gtg atg act gtt 2101
 Glu Pro Arg Ser Gly Ile Asp Thr Asn Ala Tyr Tyr Val Met Thr Val
 385 390 395

gga aca aag acg ttc ttg gtc cat cgt gag tgg ttc atg gac ctc aac 2149
 Gly Thr Lys Thr Phe Leu Val His Arg Glu Trp Phe Met Asp Leu Asn
 400 405 410

ctc cct tgg agc agt gct gga agt act gtg tgg agg aac aga gag acg 2197
 Leu Pro Trp Ser Ser Ala Gly Ser Thr Val Trp Arg Asn Arg Glu Thr
 415 420 425

tta atg gag ttt gag gaa cca cac gcc acg aag cag tct gtg ata gca 2245
 Leu Met Glu Phe Glu Glu Pro His Ala Thr Lys Gln Ser Val Ile Ala
 430 435 440 445

ttg ggc tca caa gag gga gct ctg cat caa gct ttg gct gga gcc att 2293
 Leu Gly Ser Gln Glu Gly Ala Leu His Gln Ala Leu Ala Gly Ala Ile
 450 455 460

cct gtg gaa ttt tca agc aac act gtc aag ttg acg tcg ggt cat ttg 2341
 Pro Val Glu Phe Ser Ser Asn Thr Val Lys Leu Thr Ser Gly His Leu
 465 470 475

aag tgt aga gtg aag atg gaa aaa ttg cag ttg aag gga aca acc tat 2389
 Lys Cys Arg Val Lys Met Glu Lys Leu Gln Leu Lys Gly Thr Thr Tyr
 480 485 490

ggc gtc tgt tca aag gct ttc aag ttt ctt ggg act ccc gcg gac aca 2437
 Gly Val Cys Ser Lys Ala Phe Lys Phe Leu Gly Thr Pro Ala Asp Thr
 495 500 505

ggt cac ggc act gtg gtg ttg gaa ttg cag tac act ggc acg gat gga 2485
 Gly His Gly Thr Val Val Leu Glu Leu Gln Tyr Thr Gly Thr Asp Gly
 510 515 520 525

cct tgc aaa gtt cct atc tcg tca gtg gct tca ttg aac gac cta acg 2533
 Pro Cys Lys Val Pro Ile Ser Ser Val Ala Ser Leu Asn Asp Leu Thr
 530 535 540

cca gtg ggc aga ttg gtc act gtc aac cct ttt gtt tca gtg gcc acg 2581
 Pro Val Gly Arg Leu Val Thr Val Asn Pro Phe Val Ser Val Ala Thr
 545 550 555

gcc aac gct aag gtc ctg att gaa ttg gaa cca ccc ttt gga gac tca 2629
 Ala Asn Ala Lys Val Leu Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser
 560 565 570

tac ata gtg gtg ggc aga gga gaa caa cag atc aat cac cat tgg cac 2677
 Tyr Ile Val Val Gly Arg Gly Glu Gln Gln Ile Asn His His Trp His
 575 580 585

000000-010401

aag tct gga agc agc att ggc aaa gcc ttt aca acc acc ctc aaa gga 2725
 Lys Ser Gly Ser Ser Ile Gly Lys Ala Phe Thr Thr Thr Leu Lys Gly
 590 595 600 605

gcg cag aga cta gcc gct cta gga gac aca gct tgg gac ttt gga tca 2773
 Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser
 610 615 620

gtt gga ggg gtg ttc acc tca gtt ggg aag gct gtc cat caa gtg ttc 2821
 Val Gly Gly Val Phe Thr Ser Val Gly Lys Ala Val His Gln Val Phe
 625 630 635

gga gga gca ttc cgc tca ctg ttc gga ggc atg tcc tgg ata acg caa 2869
 Gly Gly Ala Phe Arg Ser Leu Phe Gly Gly Met Ser Trp Ile Thr Gln
 640 645 650

gga ttg ctg ggg gct ctc ctg ttg tgg atg ggc atc aat gct cgt gat 2917
 Gly Leu Leu Gly Ala Leu Leu Leu Trp Met Gly Ile Asn Ala Arg Asp
 655 660 665

agg tcc ata gct ctc acg ttt ctc gca gtt gga gga gtt ctg ctc ttc 2965
 Arg Ser Ile Ala Leu Thr Phe Leu Ala Val Gly Gly Val Leu Leu Phe
 670 675 680 685

ctc tcc gtg aac gtg cac gcc t gaaggcggcc gctcgagcat gcatctagag 3017
 Leu Ser Val Asn Val His Ala
 690

ggccctattc tatagtgtca cctaaatgct agagctcgct gatcagcctc gactgtgcct 3077
 tctagttgcc agccatctgt tgtttgcccc tcccccgctc cttccttgac cctggaagggt 3137
 gccactccca ctgtccttcc ctaataaaat gaggaatttg catcgatttg tctgagtagg 3197
 tgtcattcta ttctgggggg tgggggtggg caggacagca agggggagga ttgggaagac 3257
 aatagcaggg atgctgggga tgcgggtgggc tctatggctt ctgaggcgga aagaaccagc 3317
 tgcattaatg aatcgcccaa cgcgcgggga gaggcgggtt gcgtattggg cgctcttccg 3377
 cttcctcgct cactgactcg ctgcgctcgg tcgttcgggt gcggcgagcg gtatcagctc 3437
 actcaaaggc ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt 3497
 gagcaaaagg ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc 3557
 ataggctccg cccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa 3617
 acccgacagg actataaaga taccaggcgt ttccccctgg aagctccctc gtgcgctctc 3677
 ctgttccgac cctgccgctt accggatacc tgtccgcctt tctcccttcg ggaagcgtgg 3737
 cgctttctca tagctcacgc tgtaggtatc tcagttcggg ttaggtcggt cgctccaagc 3797
 tgggctgtgt gcacgaaccc cccgttcacg ccgaccgctg cgccttatcc ggtaactatc 3857
 gtcttgagtc caaccgggta agacacgact tatcgccact ggcagcagcc actggtaaca 3917
 ggattagcag agcgagggtat gtaggcgggt ctacagagtt cttgaagtgg tggcctaact 3977
 acggctacac tagaagaaca gtatttggtat tctgcgctct gctgaagcca gttaccttcg 4037
 gaaaaagagt tggtagctct tgatccggca aacaaaccac cgctggtagc ggtgggtttt 4097
 ttgtttgcaa gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct 4157
 tttctacggg gtctgacgct cagtggaaag aaaactcacg ttaagggatt ttgggtcatga 4217
 gattatcaaa aaggatcttc acctagatcc ttttaaatga aaaatgaagt tttaaatcaa 4277
 tctaaagtat atatgagtaa acttggtctg acagttacca atgcttaatc agtgaggcac 4337
 ctatctcagc gatctgtcta ttctgttcga ccatagttgc ctgactcccc gtcgtgtaga 4397
 taactacgat acgggagggc ttaccatctg gccccagtc tgcaatgata ccgcgagacc 4457
 cacgctcacc ggctccagat ttatcagcaa taaaccagcc agccggaagg gcgcgagcga 4517

000000-5792260

[illegible]

<400> 16															
Met	Gly	Lys	Arg	Ser	Ala	Gly	Ser	Ile	Met	Trp	Leu	Ala	Ser	Leu	Ala
1				5					10					15	
Val	Val	Ile	Ala	Cys	Ala	Gly	Ala	Val	Thr	Leu	Ser	Asn	Phe	Gln	Gly
			20					25					30		
Lys	Val	Met	Met	Thr	Val	Asn	Ala	Thr	Asp	Val	Thr	Asp	Val	Ile	Thr
		35					40					45			
Ile	Pro	Thr	Ala	Ala	Gly	Lys	Asn	Leu	Cys	Ile	Val	Arg	Ala	Met	Asp
	50					55					60				
Val	Gly	Tyr	Met	Cys	Asp	Asp	Thr	Ile	Thr	Tyr	Glu	Cys	Pro	Val	Leu
65					70					75					80
Ser	Ala	Gly	Asn	Asp	Pro	Glu	Asp	Ile	Asp	Cys	Trp	Cys	Thr	Lys	Ser
			85					90						95	
Ala	Val	Tyr	Val	Arg	Tyr	Gly	Arg	Cys	Thr	Lys	Thr	Arg	His	Ser	Arg
			100					105					110		
Arg	Ser	Arg	Arg	Ser	Leu	Thr	Val	Gln	Thr	His	Gly	Glu	Ser	Thr	Leu
		115					120					125			
Ala	Asn	Lys	Lys	Gly	Ala	Trp	Met	Asp	Ser	Thr	Lys	Ala	Thr	Arg	Tyr
	130					135					140				
Leu	Val	Lys	Thr	Glu	Ser	Trp	Ile	Leu	Arg	Asn	Pro	Gly	Tyr	Ala	Leu
145					150					155					160
Val	Ala	Ala	Val	Ile	Gly	Trp	Met	Leu	Gly	Ser	Asn	Thr	Met	Gln	Arg
			165							170				175	
Val	Val	Phe	Val	Val	Leu	Leu	Leu	Leu	Val	Ala	Pro	Ala	Tyr	Ser	Phe
		180						185					190		
Asn	Cys	Leu	Gly	Met	Ser	Asn	Arg	Asp	Phe	Leu	Glu	Gly	Val	Ser	Gly
	195						200					205			

Ala Thr Trp Val Asp Leu Val Leu Glu Gly Asp Ser Cys Val Thr Ile
 210 215 220
 Met Ser Lys Asp Lys Pro Thr Ile Asp Val Lys Met Met Asn Met Glu
 225 230 235 240
 Ala Ala Asn Leu Ala Glu Val Arg Ser Tyr Cys Tyr Leu Ala Thr Val
 245 250 255
 Ser Asp Leu Ser Thr Lys Ala Ala Cys Pro Thr Met Gly Glu Ala His
 260 265 270
 Asn Asp Lys Arg Ala Asp Pro Ala Phe Val Cys Arg Gln Gly Val Val
 275 280 285
 Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser Ile
 290 295 300
 Asp Thr Cys Ala Lys Phe Ala Cys Ser Thr Lys Ala Ile Gly Arg Thr
 305 310 315 320
 Ile Leu Lys Glu Asn Ile Lys Tyr Glu Val Ala Ile Phe Val His Gly
 325 330 335
 Pro Thr Thr Val Glu Ser His Gly Asn Tyr Ser Thr Gln Val Gly Ala
 340 345 350
 Thr Gln Ala Gly Arg Phe Ser Ile Thr Pro Ala Ala Pro Ser Tyr Thr
 355 360 365
 Leu Lys Leu Gly Glu Tyr Gly Glu Val Thr Val Asp Cys Glu Pro Arg
 370 375 380
 Ser Gly Ile Asp Thr Asn Ala Tyr Tyr Val Met Thr Val Gly Thr Lys
 385 390 395 400
 Thr Phe Leu Val His Arg Glu Trp Phe Met Asp Leu Asn Leu Pro Trp
 405 410 415
 Ser Ser Ala Gly Ser Thr Val Trp Arg Asn Arg Glu Thr Leu Met Glu
 420 425 430
 Phe Glu Glu Pro His Ala Thr Lys Gln Ser Val Ile Ala Leu Gly Ser
 435 440 445
 Gln Glu Gly Ala Leu His Gln Ala Leu Ala Gly Ala Ile Pro Val Glu
 450 455 460
 Phe Ser Ser Asn Thr Val Lys Leu Thr Ser Gly His Leu Lys Cys Arg
 465 470 475 480
 Val Lys Met Glu Lys Leu Gln Leu Lys Gly Thr Thr Tyr Gly Val Cys
 485 490 495
 Ser Lys Ala Phe Lys Phe Leu Gly Thr Pro Ala Asp Thr Gly His Gly
 500 505 510
 Thr Val Val Leu Glu Leu Gln Tyr Thr Gly Thr Asp Gly Pro Cys Lys
 515 520 525
 Val Pro Ile Ser Ser Val Ala Ser Leu Asn Asp Leu Thr Pro Val Gly
 530 535 540
 Arg Leu Val Thr Val Asn Pro Phe Val Ser Val Ala Thr Ala Asn Ala
 545 550 555 560
 Lys Val Leu Ile Glu Leu Glu Pro Pro Phe Gly Asp Ser Tyr Ile Val
 565 570 575
 Val Gly Arg Gly Glu Gln Gln Ile Asn His His Trp His Lys Ser Gly
 580 585 590
 Ser Ser Ile Gly Lys Ala Phe Thr Thr Thr Leu Lys Gly Ala Gln Arg
 595 600 605
 Leu Ala Ala Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Val Gly Gly
 610 615 620
 Val Phe Thr Ser Val Gly Lys Ala Val His Gln Val Phe Gly Gly Ala
 625 630 635 640

TODD ST 92360

Phe Arg Ser Leu Phe Gly Gly Met Ser Trp Ile Thr Gln Gly Leu Leu
645 650 655
Gly Ala Leu Leu Leu Trp Met Gly Ile Asn Ala Arg Asp Arg Ser Ile
660 665 670
Ala Leu Thr Phe Leu Ala Val Gly Gly Val Leu Leu Phe Leu Ser Val
675 680 685
Asn Val His Ala
690

<210> 17
<211> 5334
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of artificial sequence; note =
synthetic construct

<221> CDS
<222> (916)...(3007)
<221> misc_feature
<222> (1)...(5334)
<223> pCBE 1-14

<400> 17
gacggatcgg gagatctccc gatccccctat ggtgcactct cagtacaatc tgctctgatg 60
ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg 120
cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180
ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt 240
gattattgac tagttattaa tagtaataaa ttacggggtc attagttcat agcccatata 300
tggagttccg cgttacataa cttacggtaa atggcccccc tggctgaccg cccaacgacc 360
cccgccatt gagctcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
attgacgtca atgggtggag tatttacggt aaactgcca cttggcagta catcaagtgt 480
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540
atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca 600
tcgctattac catggtgatg cgggttttggc agtacatcaa tgggcggtga tagcgggttg 660
actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720
aaaatcaacg ggactttcca aaatgtcgta acaactccgc ccattgacg caaatgggcg 780
gtaggcggtg acggtgggag gtctatataa gcagagctct ctggctaact agagaaccca 840
ctgcttactg gcttatcgaa attaatacga ctactatag ggagacccaa gcttgggtacc 900
tctagagccg ccgcc atg ggc aga aag caa aac aaa aga gga gga aat gaa 951
Met Gly Arg Lys Gln Asn Lys Arg Gly Gly Asn Glu
1 5 10

ggc tca atc atg tgg ctc gcg agc ttg gca gtt gtc ata gct tgt gcg 999
Gly Ser Ile Met Trp Leu Ala Ser Leu Ala Val Val Ile Ala Cys Ala
15 20 25

gga gcc atg aag ttg tcg aat ttc cag ggg aag ctt ttg atg acc atc 1047
Gly Ala Met Lys Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile
30 35 40

0982615-010401

aac aac acg gac att gca gac gtt atc gtg att ccc acc tca aaa gga Asn Asn Thr Asp Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly 45 50 55 60	1095
gag aac aga tgc tgg gtc cgg gca atc gac gtc ggc tac atg tgt gag Glu Asn Arg Cys Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu 65 70 75	1143
gac act atc acg tac gaa tgt cct aag ctt acc atg ggc aat gat cca Asp Thr Ile Thr Tyr Glu Cys Pro Lys Leu Thr Met Gly Asn Asp Pro 80 85 90	1191
gag gat gtg gat tgc tgg tgt gac aac caa gaa gtc tac gtc caa tat Glu Asp Val Asp Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr 95 100 105	1239
gga cgg tgc acg cgg acc agg cat tcc aag cga agc agg aga tcc gtg Gly Arg Cys Thr Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val 110 115 120	1287
tcg gtc caa aca cat ggg gag agt tca cta gtg aat aaa aaa gag gct Ser Val Gln Thr His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala 125 130 135 140	1335
tgg ctg gat tca acg aaa gcc aca cga tat ctc atg aaa act gag aac Trp Leu Asp Ser Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn 145 150 155	1383
tgg atc ata agg aat cct ggc tat gct ttc ctg gcg gcg gta ctt ggc Trp Ile Ile Arg Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly 160 165 170	1431
tgg atg ctt ggc agt aac aac ggt caa cgc gtg gta ttt acc atc ctc Trp Met Leu Gly Ser Asn Asn Gly Gln Arg Val Val Phe Thr Ile Leu 175 180 185	1479
ctg ctg ttg gtc gct ccg gct tac agt ttt aat tgt ctg gga atg ggc Leu Leu Leu Val Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly 190 195 200	1527
aat cgt gac ttc ata gaa gga gcc agt gga gcc act tgg gtg gac ttg Asn Arg Asp Phe Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu 205 210 215 220	1575
gtg ctg gaa gga gat agc tgc ttg aca atc atg gca aac gac aaa cca Val Leu Glu Gly Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro 225 230 235	1623
aca ttg gac gtc cgc atg att aac atc gaa gct agc caa ctt gct gag Thr Leu Asp Val Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu 240 245 250	1671
gtc aga agt tac tgc tat cat gct tca gtc act gac atc tcg acg gtg	1719

Val	Arg	Ser	Tyr	Cys	Tyr	His	Ala	Ser	Val	Thr	Asp	Ile	Ser	Thr	Val																	
255																260	265															
gct	cgg	tgc	ccc	acg	act	gga	gaa	gcc	cac	aac	gag	aag	cga	gct	gat	1767																
Ala	Arg	Cys	Pro	Thr	Thr	Gly	Glu	Ala	His	Asn	Glu	Lys	Arg	Ala	Asp																	
270																275	280															
agt	agc	tat	gtg	tgc	aaa	caa	ggc	ttc	act	gac	cgt	ggg	tgg	ggc	aac	1815																
Ser	Ser	Tyr	Val	Cys	Lys	Gln	Gly	Phe	Thr	Asp	Arg	Gly	Trp	Gly	Asn																	
285																290	295															
gga	tgt	gga	ctt	ttc	ggg	aag	gga	agc	att	gac	aca	tgt	gca	aaa	ttc	1863																
Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser	Ile	Asp	Thr	Cys	Ala	Lys	Phe																	
305																310	315															
tcc	tgc	acc	agt	aaa	gcg	att	ggg	aga	aca	atc	cag	cca	gaa	aac	atc	1911																
Ser	Cys	Thr	Ser	Lys	Ala	Ile	Gly	Arg	Thr	Ile	Gln	Pro	Glu	Asn	Ile																	
320																325	330															
aaa	tac	gaa	gtt	ggc	att	ttt	gtg	cat	gga	acc	acc	act	tcg	gaa	aac	1959																
Lys	Tyr	Glu	Val	Gly	Ile	Phe	Val	His	Gly	Thr	Thr	Thr	Ser	Glu	Asn																	
335																340	345															
cat	ggg	aat	tat	tca	gcg	caa	gtt	ggg	gcg	tcc	cag	gcg	gca	aag	ttt	2007																
His	Gly	Asn	Tyr	Ser	Ala	Gln	Val	Gly	Ala	Ser	Gln	Ala	Ala	Lys	Phe																	
350																355	360															
aca	gta	aca	ccc	aat	gct	cct	tcg	ata	acc	ctc	aaa	ctt	ggt	gac	tac	2055																
Thr	Val	Thr	Pro	Asn	Ala	Pro	Ser	Ile	Thr	Leu	Lys	Leu	Gly	Asp	Tyr																	
365																370	375															
gga	gaa	gtc	aca	ctg	gac	tgt	gag	cca	agg	agt	gga	ctg	aac	act	gaa	2103																
Gly	Glu	Val	Thr	Leu	Asp	Cys	Glu	Pro	Arg	Ser	Gly	Leu	Asn	Thr	Glu																	
385																390	395															
gcg	ttt	tac	gtc	atg	acc	gtg	ggg	tca	aag	tca	ttt	ctg	gtc	cat	agg	2151																
Ala	Phe	Tyr	Val	Met	Thr	Val	Gly	Ser	Lys	Ser	Phe	Leu	Val	His	Arg																	
400																405	410															
gag	tgg	ttt	cat	gac	ctc	gct	ctc	ccc	tgg	acg	tcc	cct	tcg	agc	aca	2199																
Glu	Trp	Phe	His	Asp	Leu	Ala	Leu	Pro	Trp	Thr	Ser	Pro	Ser	Ser	Thr																	
415																420	425															
gcg	tgg	aga	aac	aga	gaa	ctc	ctc	atg	gaa	ttt	gaa	gag	gcg	cac	gcc	2247																
Ala	Trp	Arg	Asn	Arg	Glu	Leu	Leu	Met	Glu	Phe	Glu	Glu	Ala	His	Ala																	
430																435	440															
aca	aaa	cag	tcc	gtt	gtt	gct	ctt	ggg	tca	cag	gaa	gga	ggc	ctc	cat	2295																
Thr	Lys	Gln	Ser	Val	Val	Ala	Leu	Gly	Ser	Gln	Glu	Gly	Gly	Leu	His																	
445																450	455															
cag	gcg	ttg	gca	gga	gcc	atc	gtg	gtg	gag	tac	tca	agc	tca	gtg	aag	2343																
Gln	Ala	Leu	Ala	Gly	Ala	Ile	Val	Val	Glu	Tyr	Ser	Ser	Ser	Val	Lys																	
465																470	475															

[illegible]

tta aca tca ggc cac ctg aaa tgt agg ctg aaa atg gac aaa ctg gct Leu Thr Ser Gly His Leu Lys Cys Arg Leu Lys Met Asp Lys Leu Ala 480 485 490	2391
ctg aaa ggc aca acc tat ggc atg tgt aca gaa aaa ttc tgc ttc gcg Leu Lys Gly Thr Thr Tyr Gly Met Cys Thr Glu Lys Phe Ser Phe Ala 495 500 505	2439
aaa aat ccg gcg gac act ggt cac gga aca gtt gtc att gaa ctc tcc Lys Asn Pro Ala Asp Thr Gly His Gly Thr Val Val Ile Glu Leu Ser 510 515 520	2487
tac tct ggg agt gat ggc ccc tgc aaa att ccg att gct tcc gtt gcg Tyr Ser Gly Ser Asp Gly Pro Cys Lys Ile Pro Ile Ala Ser Val Ala 525 530 535 540	2535
agc ctc aat gac atg acc ccc gtt ggg cgg ctg gtg aca gtg aac ccc Ser Leu Asn Asp Met Thr Pro Val Gly Arg Leu Val Thr Val Asn Pro 545 550 555	2583
ttc gtc gcg act tcc agt gcc agc tca aag gtg ctg gtc gag atg gaa Phe Val Ala Thr Ser Ser Ala Ser Ser Lys Val Leu Val Glu Met Glu 560 565 570	2631
ccc ccc ttc gga gac tcc tac atc gta gtt gga agg gga gac aag cag Pro Pro Phe Gly Asp Ser Tyr Ile Val Val Gly Arg Gly Asp Lys Gln 575 580 585	2679
atc aac cac cat tgg cac aaa gct gga agc acg ctg ggc aag gcc ttt Ile Asn His His Trp His Lys Ala Gly Ser Thr Leu Gly Lys Ala Phe 590 595 600	2727
tca aca act ttg aag gga gct caa aga ctg gca gcg ttg ggc gac aca Ser Thr Thr Leu Lys Gly Ala Gln Arg Leu Ala Ala Leu Gly Asp Thr 605 610 615 620	2775
gcc tgg gac ttt ggc tct att gga ggg gtc ttc aac tcc ata gga aaa Ala Trp Asp Phe Gly Ser Ile Gly Gly Val Phe Asn Ser Ile Gly Lys 625 630 635	2823
gcc gtt cac caa gtg ttt ggt ggt gcc ttc aga aca ctc ttt ggg gga Ala Val His Gln Val Phe Gly Gly Ala Phe Arg Thr Leu Phe Gly Gly 640 645 650	2871
atg tct tgg atc aca caa ggg cta atg ggt gcc cta ctg ctc tgg atg Met Ser Trp Ile Thr Gln Gly Leu Met Gly Ala Leu Leu Leu Trp Met 655 660 665	2919
ggc gtc aac gca cga gac cga tca att gct ttg gcc ttc tta gcc aca Gly Val Asn Ala Arg Asp Arg Ser Ile Ala Leu Ala Phe Leu Ala Thr 670 675 680	2967
ggg ggt gtg ctc gtg ttc tta gcg acc aat gtg cat gct t aattagtttg	3017

Gly Gly Val Leu Val Phe Leu Ala Thr Asn Val His Ala
685 690 695

agcggcgcgt cgagcatgca tctagagggc cctattctat agtgtcacct aaatgctaga 3077
gctcgctgat cagcctcgac tgtgccttct agttgccagc catctgttgt ttgccccctc 3137
cccgtgcctt ccttgaccct ggaaggtgcc actcccactg tcccttccta ataaaatgag 3197
gaaattgcat cgcattgtct gagtagtgtt cattctattc tgggggggtg ggtggggcag 3257
gacagcaagg gggaggattg ggaagacaat agcaggcatg ctgggggatg ggtgggctct 3317
atggcttctg aggcggaaag aaccagctgc attaatgaat cggccaacgc gcggggagag 3377
gcggtttgct tattgggcgc tcttcgcctt cctcgctcac tgactcgctg cgtcggtcg 3437
ttcggctgct gcgagcggta tcagctcact caaaggcggg aatacgggta tccacagaat 3497
caggggataa cgcaggaaag aacatgtgag caaaaggcca gcaaaaggcc aggaaccgta 3557
aaaaggccgc gttgctggcg tttttccata ggctccgccc cctgacgag catcacaaaa 3617
atcgacgctc aagtcagagg tggcgaaacc cgacaggact ataaagatac caggcggttc 3677
cccctggaag ctccctcgct cgtctcctg ttccgacctt gccgcttacc ggatacctgt 3737
ccgcctttct cccttcggga agcgtggcgc tttctcatag ctacgctgt aggtatctca 3797
gttcggtgta ggtcgctgc tccaagctgg gctgtgtgca cgaaccccc gttcagccc 3857
accgctgctg cttatccggt aactatcgct ttgagtccta cccggtaaga cagcacttat 3917
cgccactggc agcagccact ggtaacagga ttagcagagc gaggtatgta ggcggtgcta 3977
cagagttctt gaagtgggtg cctaactacg gctacactag aagaacagta ttggtatct 4037
gcgctctgct gaagccagtt accttcggaa aaagagttgg tagctcttga tccggcaaac 4097
aaaccaccgc tggtagcggg ggtttttttt tttgcaagca gcagattacg cgcagaaaaa 4157
aaggatctca agaagatcct ttgatctttt ctacgggggtc tgacgctcag tggaaacgaa 4217
actcacgtta agggattttt gtcatgagat tatcaaaaag gatcttcacc tagatccttt 4277
taaattaaaa atgaagtttt aaatcaatct aaagtatata tgagtaaact tgggtctgaca 4337
gttaccaatg cttaatcagt gaggcacctt tctcagcgat ctgtctatct cgttcatcca 4397
tagttgcctg actccccgct gtgtagataa ctacgatacg ggagggtta ccatctggcc 4457
ccagtgcctg aatgataccg cgagaccac gctcacggc tccagattta tcagcaataa 4517
accagccagc cggaagggcc gagcgcagaa gtggtcctgc aactttatcc gcctccatcc 4577
agtctattaa ttggtgccgg gaagctagag taagtagttc gccagttaat agtttgcgca 4637
acgttgctgc cattgctaca ggcatcgtgg tgcacgctc gtcgtttggt atggcttcat 4697
tcagctccgg tcccaacga tcaaggcgag ttacatgatc ccccatgttg tgcaaaaaag 4757
cgggttagctc cttcggtcct ccgatcgttg tcagaagtaa gttggccgca gtgttatcac 4817
tcattggtat ggcagcactg cataattctc ttactgtcat gccatccgta agatgctttt 4877
ctgtgactgg tgagtactca accaagtcct tctgagaata gtgtatgcgg cgaccgagtt 4937
gctcttgccc ggcgtcaata cgggataata ccgcgccaca tagcagaact ttaaaagtgc 4997
tcatcattgg aaaacgttct tcggggcgaa aactctcaag gatcttaccg ctgttgagat 5057
ccagttcgat gtaaccact cgtgcaacca actgatcttc agcatctttt actttcacca 5117
gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc aaaaaaggga ataaggcgca 5177
cacggaaatg ttgaatactc atactcttcc tttttcaata ttattgaagc atttatcagg 5237
gttattgtct catgagcgga tacatatattg aatgtattta gaaaaataaa caaatagggg 5297
ttccgpcac atttccccga aaagtgccac ctgacgt 5334

<210> 18

<211> 697

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
synthetic construct

<223> pCBE 1-14

098645:040404

<400> 18

Met Gly Arg Lys Gln Asn Lys Arg Gly Gly Asn Glu Gly Ser Ile Met
1 5 10 15
Trp Leu Ala Ser Leu Ala Val Val Ile Ala Cys Ala Gly Ala Met Lys
20 25 30
Leu Ser Asn Phe Gln Gly Lys Leu Leu Met Thr Ile Asn Asn Thr Asp
35 40 45
Ile Ala Asp Val Ile Val Ile Pro Thr Ser Lys Gly Glu Asn Arg Cys
50 55 60
Trp Val Arg Ala Ile Asp Val Gly Tyr Met Cys Glu Asp Thr Ile Thr
65 70 75 80
Tyr Glu Cys Pro Lys Leu Thr Met Gly Asn Asp Pro Glu Asp Val Asp
85 90 95
Cys Trp Cys Asp Asn Gln Glu Val Tyr Val Gln Tyr Gly Arg Cys Thr
100 105 110
Arg Thr Arg His Ser Lys Arg Ser Arg Arg Ser Val Ser Val Gln Thr
115 120 125
His Gly Glu Ser Ser Leu Val Asn Lys Lys Glu Ala Trp Leu Asp Ser
130 135 140
Thr Lys Ala Thr Arg Tyr Leu Met Lys Thr Glu Asn Trp Ile Ile Arg
145 150 155 160
Asn Pro Gly Tyr Ala Phe Leu Ala Ala Val Leu Gly Trp Met Leu Gly
165 170 175
Ser Asn Asn Gly Gln Arg Val Val Phe Thr Ile Leu Leu Leu Leu Val
180 185 190
Ala Pro Ala Tyr Ser Phe Asn Cys Leu Gly Met Gly Asn Arg Asp Phe
195 200 205
Ile Glu Gly Ala Ser Gly Ala Thr Trp Val Asp Leu Val Leu Glu Gly
210 215 220
Asp Ser Cys Leu Thr Ile Met Ala Asn Asp Lys Pro Thr Leu Asp Val
225 230 235 240
Arg Met Ile Asn Ile Glu Ala Ser Gln Leu Ala Glu Val Arg Ser Tyr
245 250 255
Cys Tyr His Ala Ser Val Thr Asp Ile Ser Thr Val Ala Arg Cys Pro
260 265 270
Thr Thr Gly Glu Ala His Asn Glu Lys Arg Ala Asp Ser Ser Tyr Val
275 280 285
Cys Lys Gln Gly Phe Thr Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu
290 295 300
Phe Gly Lys Gly Ser Ile Asp Thr Cys Ala Lys Phe Ser Cys Thr Ser
305 310 315 320
Lys Ala Ile Gly Arg Thr Ile Gln Pro Glu Asn Ile Lys Tyr Glu Val
325 330 335
Gly Ile Phe Val His Gly Thr Thr Thr Ser Glu Asn His Gly Asn Tyr
340 345 350
Ser Ala Gln Val Gly Ala Ser Gln Ala Ala Lys Phe Thr Val Thr Pro
355 360 365
Asn Ala Pro Ser Ile Thr Leu Lys Leu Gly Asp Tyr Gly Glu Val Thr
370 375 380
Leu Asp Cys Glu Pro Arg Ser Gly Leu Asn Thr Glu Ala Phe Tyr Val
385 390 395 400
Met Thr Val Gly Ser Lys Ser Phe Leu Val His Arg Glu Trp Phe His
405 410 415

F01010-S112360

[illegible]

gattattgac tagttattaa tagtaataca ttacgggggc attagttcat agcccatata 300
 tggagttccg cgttacataa cttacggtaa atggcccgcg tggtgaccg cccaacgacc 360
 cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
 attgacgtca atgggtggac tatttacggt aaactgcccc cttggcagta catcaagtgt 480
 atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540
 atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca 600
 tcgctattac catggtgatg cggttttggc agtacatcaa tggcggtgga tagcggtttg 660
 actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720
 aaaatcaacg ggactttcca aaatgtcgta acaactccgc cccattgacg caaatgggag 780
 gtaggcgtgt acggtgggag gtctatataa gcagagctct ctggctaact agagaaccca 840
 ctgcttactg gcttatcgaa attaatacga ctactatag ggagaccгаа gcttggtacc 900
 gccgccgcc atg ggc aag agg tcc gcc ggc tca atc atg tgg ctc gcg agc 951
 Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser
 1 5 10

ttg gca gtt gtc ata gct ggt aca agc gct acc acc atc cac cgg gac 999
 Leu Ala Val Val Ile Ala Gly Thr Ser Ala Thr Thr Ile His Arg Asp
 15 20 25 30

agg gaa gga tac atg gtt atg cgg gcc agt gga agg gac gct gca agc 1047
 Arg Glu Gly Tyr Met Val Met Arg Ala Ser Gly Arg Asp Ala Ala Ser
 35 40 45

cag gtc agg gta caa aac gga acg tgc gtc atc ctg gca aca gac atg 1095
 Gln Val Arg Val Gln Asn Gly Thr Cys Val Ile Leu Ala Thr Asp Met
 50 55 60

gga gag tgg tgt gaa gat tca atc acc tac tct tgc gtc acg att gac 1143
 Gly Glu Trp Cys Glu Asp Ser Ile Thr Tyr Ser Cys Val Thr Ile Asp
 65 70 75

cag gag gaa gaa ccc gtt gac gtg gac tgc ttc tgc cga ggt gtt gat 1191
 Gln Glu Glu Glu Pro Val Asp Val Asp Cys Phe Cys Arg Gly Val Asp
 80 85 90

agg gtt aag tta gag tat gga cgc tgt gga agg caa gct gga tct agg 1239
 Arg Val Lys Leu Glu Tyr Gly Arg Cys Gly Arg Gln Ala Gly Ser Arg
 95 100 105 110

ggg aaa agg tct gtg gtc att cca aca cat gca caa aaa gac atg gtc 1287
 Gly Lys Arg Ser Val Val Ile Pro Thr His Ala Gln Lys Asp Met Val
 115 120 125

ggg cga ggt cat gca tgg ctt aaa ggt gac aat att cga gat cat gtc 1335
 Gly Arg Gly His Ala Trp Leu Lys Gly Asp Asn Ile Arg Asp His Val
 130 135 140

acc cga gtc gag ggc tgg atg tgg aag aac aag ctt cta act gcc gcc 1383
 Thr Arg Val Glu Gly Trp Met Trp Lys Asn Lys Leu Leu Thr Ala Ala
 145 150 155

att gtg gcc ttg gct tgg ctc atg gtt gat agt tgg atg gcc aga gtg 1431
 Ile Val Ala Leu Ala Trp Leu Met Val Asp Ser Trp Met Ala Arg Val
 160 165 170

T 04070-51192800

act gtc atc ctc ttg gcg ttg agt cta ggg cca gtg tac gcc acg agg 1479
 Thr Val Ile Leu Leu Ala Leu Ser Leu Gly Pro Val Tyr Ala Thr Arg
 175 180 185 190

tgc acg cat ctt gag aac aga gat ttt gtg aca gga act caa ggg acc 1527
 Cys Thr His Leu Glu Asn Arg Asp Phe Val Thr Gly Thr Gln Gly Thr
 195 200 205

acc aga gtg tcc cta gtt ttg gaa ctt gga ggc tgc gtg acc atc aca 1575
 Thr Arg Val Ser Leu Val Leu Glu Leu Gly Gly Cys Val Thr Ile Thr
 210 215 220

gct gag ggc aag cca tcc att gat gta tgg ctc gaa gac att ttt cag 1623
 Ala Glu Gly Lys Pro Ser Ile Asp Val Trp Leu Glu Asp Ile Phe Gln
 225 230 235

gaa agc ccg gct gaa acc aga gaa tac tgc ctg cac gcc aaa ttg acc 1671
 Glu Ser Pro Ala Glu Thr Arg Glu Tyr Cys Leu His Ala Lys Leu Thr
 240 245 250

aac aca aaa gtg gag gct cgc tgt cca acc act gga ccg gcg aca ctt 1719
 Asn Thr Lys Val Glu Ala Arg Cys Pro Thr Thr Gly Pro Ala Thr Leu
 255 260 265 270

ccg gag gag cat cag gct aat atg gtg tgc aag aga gac caa agc gac 1767
 Pro Glu Glu His Gln Ala Asn Met Val Cys Lys Arg Asp Gln Ser Asp
 275 280 285

cgt gga tgg gga aac cac tgc ggg ttt ttt ggg aag ggc agt ata gtg 1815
 Arg Gly Trp Gly Asn His Cys Gly Phe Phe Gly Lys Gly Ser Ile Val
 290 295 300

gct tgt gca aag ttt gaa tgc gag gaa gca aaa aaa gct gtg ggc cac 1863
 Ala Cys Ala Lys Phe Glu Cys Glu Glu Ala Lys Lys Ala Val Gly His
 305 310 315

gtc tat gac tcc aca aag atc acg tat gtt gtc aag gtt gag ccc cac 1911
 Val Tyr Asp Ser Thr Lys Ile Thr Tyr Val Val Lys Val Glu Pro His
 320 325 330

aca ggg gat tac ttg gct gca aat gag acc aat tca aac agg aaa tca 1959
 Thr Gly Asp Tyr Leu Ala Ala Asn Glu Thr Asn Ser Asn Arg Lys Ser
 335 340 345 350

gca cag ttt acg gtg gca tcc gag aaa gtg atc ctg cgg ctc ggc gac 2007
 Ala Gln Phe Thr Val Ala Ser Glu Lys Val Ile Leu Arg Leu Gly Asp
 355 360 365

tat gga gat gtg tgc ctg acg tgt aaa gtg gca agt ggg att gat gtc 2055
 Tyr Gly Asp Val Ser Leu Thr Cys Lys Val Ala Ser Gly Ile Asp Val
 370 375 380

gcc caa act gtg gtg atg tca ctc gac agc agc aag gac cac ctg cct 2103

FOIb015-5132860

Ala Gln Thr Val Val Met Ser Leu Asp Ser Ser Lys Asp His Leu Pro
 385 390 395

tct gca tgg caa gtg cac cgt gac tgg ttt gag gac ttg gcg ctg ccc 2151
 Ser Ala Trp Gln Val His Arg Asp Trp Phe Glu Asp Leu Ala Leu Pro
 400 405 410

tgg aaa cac aag gac aac caa gat tgg aac agt gtg gag aaa ctt gtg 2199
 Trp Lys His Lys Asp Asn Gln Asp Trp Asn Ser Val Glu Lys Leu Val
 415 420 425 430

gaa ttt gga cca cca cat gct gtg aaa atg gat gtt ttc aat ctg ggg 2247
 Glu Phe Gly Pro Pro His Ala Val Lys Met Asp Val Phe Asn Leu Gly
 435 440 445

gac cag acg gct gtg ctg ctc aaa tca ctg gca gga gtt ccg ctg gcc 2295
 Asp Gln Thr Ala Val Leu Leu Lys Ser Leu Ala Gly Val Pro Leu Ala
 450 455 460

agt gtg gag ggc cag aaa tac cac ctg aaa agc ggc cat gtt act tgt 2343
 Ser Val Glu Gly Gln Lys Tyr His Leu Lys Ser Gly His Val Thr Cys
 465 470 475

gat gtg gga ctg gaa aag ctg aaa ctg aaa ggc aca acc tac tcc atg 2391
 Asp Val Gly Leu Glu Lys Leu Lys Leu Lys Gly Thr Thr Tyr Ser Met
 480 485 490

tgt gac aaa gca aag ttc aaa tgg aag aga gtt cct gtg gac agc ggc 2439
 Cys Asp Lys Ala Lys Phe Lys Trp Lys Arg Val Pro Val Asp Ser Gly
 495 500 505 510

cat gac aca gta gtc atg gag gta tca tac aca gga agc gac aag cca 2487
 His Asp Thr Val Val Met Glu Val Ser Tyr Thr Gly Ser Asp Lys Pro
 515 520 525

tgt cgg atc ccg gtg cgg gct gtg gca cat ggt gtc cca gcg gtt aat 2535
 Cys Arg Ile Pro Val Arg Ala Val Ala His Gly Val Pro Ala Val Asn
 530 535 540

gta gcc atg ctc ata acc ccc aat cca acc att gaa aca aat ggt ggc 2583
 Val Ala Met Leu Ile Thr Pro Asn Pro Thr Ile Glu Thr Asn Gly Gly
 545 550 555

gga ttc ata gaa atg cag ctg cca cca ggg gat aac atc atc tat gtg 2631
 Gly Phe Ile Glu Met Gln Leu Pro Pro Gly Asp Asn Ile Ile Tyr Val
 560 565 570

gga gac ctt agc cag cag tgg ttt cag aaa ggc agt acc att ggt aga 2679
 Gly Asp Leu Ser Gln Gln Trp Phe Gln Lys Gly Ser Thr Ile Gly Arg
 575 580 585 590

atg ttt gaa aaa acc cgc agg gga ttg gaa agg ctc tct gtg gtt gga 2727
 Met Phe Glu Lys Thr Arg Arg Gly Leu Glu Arg Leu Ser Val Val Gly
 595 600 605

0032645-040401

gaa cat gca tgg gac ttt ggc tca gta ggc ggg gta ctg tct tct gtg Glu His Ala Trp Asp Phe Gly Ser Val Gly Gly Val Leu Ser Ser Val 610 615 620	2775
ggg aag gca atc cac acg gtg ctg ggg gga gct ttc aac acc ctt ttt Gly Lys Ala Ile His Thr Val Leu Gly Gly Ala Phe Asn Thr Leu Phe 625 630 635	2823
ggg ggg gtt gga ttc atc cct aag atg ctg ctg ggg gtt gct ctg gtc Gly Gly Val Gly Phe Ile Pro Lys Met Leu Leu Gly Val Ala Leu Val 640 645 650	2871
tgg ttg gga cta aat gcc agg aat cca acg atg tcc atg acg ttt ctt Trp Leu Gly Leu Asn Ala Arg Asn Pro Thr Met Ser Met Thr Phe Leu 655 660 665 670	2919
gct gtg ggg gct ttg aca ctg atg atg aca atg gga gtt ggg gca t Ala Val Gly Ala Leu Thr Leu Met Met Thr Met Gly Val Gly Ala 675 680 685	2965
gagcggccgc tgcagcatgc atctagaggg cccatttcta tagtgtcacc taaatgctag agctcgcgtga tcagccctcga ctgtgccttc tagttgccag ccatctgttg tttgccccctc ccccgtgcct tcccttgacc tggaagggtg cactcccact gtcctttcct aataaaatga ggaaattgca tgcattgtgc tggagtgggtg tcattctatt ctgggggggtg ggggtggggca ggacagcaag ggggaggatt gggaagacaa tagcaggcat gctggggatg cgggtgggctc tatggccttct gaggcggaaa gaacagctgc attaataaat cggccaacgc gcgggggagag gcgggtttgcg tattggggcg tcttccgctt cctcgctcac tgactcgctg cgctcggtcg ttcggctgcg gcgagcggta tcagctcact caaaggcggg aatacggtta tccacagaat caggggataa cgcaggaaaag aacatgtgag caaaaggcca gcaaaaggcc gaagaccgta aaaaggccgc gttgctggcg tttttccata ggctccgccc ccttgacgag catcacaaaa atcgacgcctc aagtcagagg tggcgaaacc cgacaggact ataaagatac caggcggttc ccccctggaag ctccctcgctg cgctctcctg ttccgaccct gccgcttacc ggatacctgt ccgcctttct cccttcggga agcgtggcg tttctcaatg ctacgctgt aggtatctca gttcgggtgta ggtcggttcgc tccaagctgg gctgtgtgca cgaaccccc gttcagcccc accgctgcgc cttatccggt aactatcgct ttgagtcgag cccggttaaga caccagcttat cgccactggc agcagccact ggtaacagga ttacagagc aaggacagta tttgggtatct cagagttctt gaagtgggtg cctaactacg gctacactag aaggacagta tttgggtatct gcgctgcgtc aagccaggtt accttcggaa aaagagttgg tagctcttga tccggcaaac aaaccaccgc tggtagcggg ggtttttttg tttgcaagca gcagattacg cgagaaaaa aaggatctca agaagatcct ttgatctttt ctacgggggtc tgacgctcag tggaacgaaa actcacgtta agggattttg gtcattgagat tatcaaaaag gatcttcacc tagatctctt taaattaaaa atgaagtttt aaatcaatct aaagtatata tgagtaaat tggctctgaca gttaccaatg cttaatcagt gaggcaccta tctcagcgat ctgtctatct cgttcatcca tagttgctcg aatccccgtc gcttagataa ctacgatacg ggagggctta ccatctggcc ccagtgcctgc actgataccg cgagaccac gctcaccggc tccagattta tcagcaataa accagccagc cggaaggggc gagcgcagaa gtggctctgc aactttatcc gctccatcc agtctattaa ttgttgccgg gaagctagag taagtgttc gccagttaat agtttgccga acgttggtgc cattgttaca ggcattcgtg tgtcacgctc gtcgtttggg atggcttcac tcagctccgg tccccaacga tcaaggcgag ttacatgac ccccatgttg tgcaaaaaag cggtagctc cttcggctct cegatcgttg tcagaagtta gttggccgca gtgttatcac tcattgggtat ggcagcactg cataattctc ttactgtcat gccatccgta agatgctttt ctgtgactgg ttgagtactca accaagtcac totgagaata gtgtatgcgg cgaccgagtt gctcttgccc ggcgtcaata cgggataata ccgcgccaca tagcagaact ttaaaagtgc	3025 3085 3145 3205 3265 3325 3385 3445 3505 3565 3625 3685 3745 3805 3865 3925 3985 4045 4105 4165 4225 4285 4345 4405 4465 4525 4585 4645 4705 4765 4825 4885 4945

tcattcattgg	aaaacgttct	tcggggcgaa	aactctcaag	gatcttaccg	ctgttgagat	5005
ccagttcgat	gtaaccact	cgtgcaccca	actgatcttc	agcatctttt	actttcacca	5065
gcgtttctgg	gtgagcaaaa	acaggaaggc	aaaatgccgc	aaaaaaggga	ataagggcga	5125
cacggaaatg	ttgaatactc	atactcttcc	tttttcaata	ttattgaagc	atttatcagg	5185
gttattgtct	catgagcgga	tacatatttg	aatgtattta	gaaaaataaa	caaatagggg	5245
ttccgcgcac	atttccccga	aaagtgccac	ctgacgtc			5283

<210> 20

<211> 681

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
synthetic construct

<400> 20

Met	Gly	Lys	Arg	Ser	Ala	Gly	Ser	Ile	Met	Trp	Leu	Ala	Ser	Leu	Ala	
1				5					10					15		
Val	Val	Ile	Ala	Gly	Thr	Ser	Ala	Val	Thr	Leu	Val	Arg	Lys	Asn	Arg	
			20					25					30			
Trp	Leu	Leu	Leu	Asn	Val	Thr	Ser	Glu	Asp	Leu	Gly	Lys	Thr	Phe	Ser	
		35					40					45				
Val	Gly	Thr	Gly	Asn	Cys	Thr	Thr	Asn	Ile	Leu	Glu	Ala	Lys	Tyr	Trp	
	50					55					60					
Cys	Pro	Asp	Ser	Met	Glu	Tyr	Asn	Cys	Pro	Asn	Leu	Ser	Pro	Arg	Glu	
65					70				75					80		
Glu	Pro	Asp	Asp	Ile	Asp	Cys	Trp	Cys	Tyr	Gly	Val	Glu	Asn	Val	Arg	
				85				90						95		
Val	Ala	Tyr	Gly	Lys	Cys	Asp	Ser	Ala	Gly	Arg	Ser	Arg	Arg	Ser	Arg	
			100					105						110		
Arg	Ala	Ile	Asp	Leu	Pro	Thr	His	Glu	Asn	His	Gly	Leu	Lys	Thr	Arg	
		115					120					125				
Gln	Glu	Lys	Trp	Met	Thr	Gly	Arg	Met	Gly	Glu	Arg	Gln	Leu	Gln	Lys	
		130				135					140					
Ile	Glu	Arg	Trp	Phe	Val	Arg	Asn	Pro	Phe	Phe	Ala	Val	Thr	Ala	Leu	
145					150				155						160	
Thr	Ile	Ala	Tyr	Leu	Val	Gly	Ser	Asn	Met	Thr	Gln	Arg	Val	Val	Ile	
			165					170						175		
Ala	Leu	Leu	Val	Leu	Ala	Val	Gly	Pro	Ala	Tyr	Ser	Ala	His	Cys	Ile	
			180					185					190			
Gly	Ile	Thr	Asp	Arg	Asp	Phe	Ile	Glu	Gly	Val	His	Gly	Gly	Thr	Trp	
	195						200					205				
Val	Ser	Ala	Thr	Leu	Glu	Gln	Asp	Lys	Cys	Val	Thr	Val	Met	Ala	Pro	
	210					215					220					
Asp	Lys	Pro	Ser	Leu	Asp	Ile	Ser	Leu	Glu	Thr	Val	Ala	Ile	Asp	Arg	
225					230				235					240		
Pro	Ala	Glu	Val	Arg	Lys	Val	Cys	Tyr	Asn	Ala	Val	Leu	Thr	His	Val	
			245					250						255		
Lys	Ile	Asn	Asp	Lys	Cys	Pro	Ser	Thr	Gly	Glu	Ala	His	Leu	Ala	Glu	
		260					265						270			
Glu	Asn	Glu	Gly	Asp	Asn	Ala	Cys	Lys	Arg	Thr	Tyr	Ser	Asp	Arg	Gly	
	275						280					285				

F01010 = SEF92300

<210> 21
<211> 5304
<212> DNA

<220>

<221> CDS

<222> (910) ... (2986)

<400> 21

[illegible]

ttg gca gtt gtc ata gct ggt aca agc gct ttg cag tta tca acc tat 999
Leu Ala Val Val Ile Ala Gly Thr Ser Ala Leu Gln Leu Ser Thr Tyr
15 20 25 30

cag ggg aaa gtg tta atg tca atc aac aag act gac gct caa agc gcc 1047
Gln Gly Lys Val Leu Met Ser Ile Asn Lys Thr Asp Ala Gln Ser Ala
35 40 45

ata aac att cct agt gcc aac gga gca aac act tgc att gtg agg gct 1095
Ile Asn Ile Pro Ser Ala Asn Gly Ala Asn Thr Cys Ile Val Arg Ala
50 55 60

cta gat gtg ggg gtc atg tgc aaa gat gac atc aca tac ctg tgc cca 1143
Leu Asp Val Gly Val Met Cys Lys Asp Asp Ile Thr Tyr Leu Cys Pro
65 70 75

gtg ctt tca gcg gga aat gat ccc gag gac att gac tgt tgg tgt gac 1191
Val Leu Ser Ala Gly Asn Asp Pro Glu Asp Ile Asp Cys Trp Cys Asp
80 85 90

gtc gaa gag gtg tgg gtg cac tac ggc aga tgc acg cgc atg gga cat 1239
Val Glu Glu Val Trp Val His Tyr Gly Arg Cys Thr Arg Met Gly His
95 100 105 110

tcc agg cgt agc cga cgg tca atc tct gtg cag cat cat gga gat tcc 1287

[illegible]

Ser Arg Arg Ser Arg Arg Ser Ile Ser Val Gln His His Gly Asp Ser
 115 120 125
 aca ctg gca aca aag aac acg cca tgg ttg gac acc gtg aaa acc acc 1335
 Thr Leu Ala Thr Lys Asn Thr Pro Trp Leu Asp Thr Val Lys Thr Thr
 130 135 140
 aaa tac ttg aca aaa gta gaa aac tgg gtt ttg cgc aat cct gga tat 1383
 Lys Tyr Leu Thr Lys Val Glu Asn Trp Val Leu Arg Asn Pro Gly Tyr
 145 150 155
 gcc cta gtt gcg ctg gcg att gga tgg atg ctc ggt agc aac aac aca 1431
 Ala Leu Val Ala Leu Ala Ile Gly Trp Met Leu Gly Ser Asn Asn Thr
 160 165 170
 cag aga gtg gtt ttt gtg atc atg ctg atg ctg att gct ccg gca tac 1479
 Gln Arg Val Val Phe Val Ile Met Leu Met Leu Ile Ala Pro Ala Tyr
 175 180 185 190
 agc ttc aac tgt ctg gga aca tca aac agg gac ttt gtc gag gga gcc 1527
 Ser Phe Asn Cys Leu Gly Thr Ser Asn Arg Asp Phe Val Glu Gly Ala
 195 200 205
 agt ggg gca aca tgg att gac ttg gta ctt gaa ggg gga agc tgt gtc 1575
 Ser Gly Ala Thr Trp Ile Asp Leu Val Leu Glu Gly Gly Ser Cys Val
 210 215 220
 aca gtg atg gca cca gag aaa cca aca ctg gac ttc aaa gtg atg aag 1623
 Thr Val Met Ala Pro Glu Lys Pro Thr Leu Asp Phe Lys Val Met Lys
 225 230 235
 atg gag gct acc gag tta gcc act gtg cgt gag tat tgt tac gaa gca 1671
 Met Glu Ala Thr Glu Leu Ala Thr Val Arg Glu Tyr Cys Tyr Glu Ala
 240 245 250
 acc ttg gac acg ctg tca aca gtg gca agg tgc ccc aca aca gga gaa 1719
 Thr Leu Asp Thr Leu Ser Thr Val Ala Arg Cys Pro Thr Thr Gly Glu
 255 260 265 270
 gct cac aac acc aaa agg agt gac cca aca ttt gtc tgc aaa aga gat 1767
 Ala His Asn Thr Lys Arg Ser Asp Pro Thr Phe Val Cys Lys Arg Asp
 275 280 285
 gtt gtg gac cgc gga tgg ggt aac gga tgt ggt ctg ttt gga aaa ggg 1815
 Val Val Asp Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly
 290 295 300
 agc att gac aca tgc gct aag ttc aca tgc aaa aac aag gca aca ggg 1863
 Ser Ile Asp Thr Cys Ala Lys Phe Thr Cys Lys Asn Lys Ala Thr Gly
 305 310 315
 aag acg atc ttg aga gaa aac atc aag tat gag gtt gca atc ttt gtg 1911
 Lys Thr Ile Leu Arg Glu Asn Ile Lys Tyr Glu Val Ala Ile Phe Val
 320 325 330

T040-573260

cat ggt tca acg gac tct acg tca cat ggc aat tac tct gag cag att 1959
 His Gly Ser Thr Asp Ser Thr Ser His Gly Asn Tyr Ser Glu Gln Ile
 335 340 345 350

gga aaa aac caa gcg gct aga ttc acc ata agc ccg caa gca ccg tcc 2007
 Gly Lys Asn Gln Ala Ala Arg Phe Thr Ile Ser Pro Gln Ala Pro Ser
 355 360 365

ttt acg gcc aac atg ggc gag tat gga aca gtt acc att gat tgt gaa 2055
 Phe Thr Ala Asn Met Gly Glu Tyr Gly Thr Val Thr Ile Asp Cys Glu
 370 375 380

gca aga tca gga atc aac acg gag gat tat tat gtt ttc act gtc aag 2103
 Ala Arg Ser Gly Ile Asn Thr Glu Asp Tyr Tyr Val Phe Thr Val Lys
 385 390 395

gag aag tca tgg cta gtg aac agg gac tgg ttt cac gac ttg aac ctt 2151
 Glu Lys Ser Trp Leu Val Asn Arg Asp Trp Phe His Asp Leu Asn Leu
 400 405 410

cca tgg acg agc cct gcc aca act gat tgg cgc aac aga gaa aca ctg 2199
 Pro Trp Thr Ser Pro Ala Thr Thr Asp Trp Arg Asn Arg Glu Thr Leu
 415 420 425 430

gtg gaa ttt gag gaa ccg cat gcc acc aag caa act gta gta gcc cta 2247
 Val Glu Phe Glu Glu Pro His Ala Thr Lys Gln Thr Val Val Ala Leu
 435 440 445

gga tcg caa gaa ggt gcc ctg cac aca gca ttg gct gga gcc att cca 2295
 Gly Ser Gln Glu Gly Ala Leu His Thr Ala Leu Ala Gly Ala Ile Pro
 450 455 460

gcc act gtt agc agc tca acc cta acc ttg caa tca ggg cat ttg aaa 2343
 Ala Thr Val Ser Ser Ser Thr Leu Thr Leu Gln Ser Gly His Leu Lys
 465 470 475

tgc aga gct aag ctt gac aag gtc aaa atc aag gga acg aca tat ggc 2391
 Cys Arg Ala Lys Leu Asp Lys Val Lys Ile Lys Gly Thr Thr Tyr Gly
 480 485 490

atg tgt gac tct gcc ttc acc ttc agc aag aac cca act gac aca ggg 2439
 Met Cys Asp Ser Ala Phe Thr Phe Ser Lys Asn Pro Thr Asp Thr Gly
 495 500 505 510

cac ggg aca gtg att gtg gaa ctg cag tat act gga agc aac gga ccc 2487
 His Gly Thr Val Ile Val Glu Leu Gln Tyr Thr Gly Ser Asn Gly Pro
 515 520 525

tgc cga gtt ccc atc tcc gtg act gca aac ctc atg gat ttg aca ccg 2535
 Cys Arg Val Pro Ile Ser Val Thr Ala Asn Leu Met Asp Leu Thr Pro
 530 535 540

gtt gga aga ttg gtc acg gtc aat ccc ttt ata agc aca ggg gga gcg 2583

00000-574330

Val	Gly	Arg	Leu	Val	Thr	Val	Asn	Pro	Phe	Ile	Ser	Thr	Gly	Gly	Ala		
545						550						555					
aac	aac	aag	gtc	atg	atc	gaa	gtt	gaa	cca	ccc	ttt	ggc	gat	tct	tac	2631	
Asn	Asn	Lys	Val	Met	Ile	Glu	Val	Glu	Pro	Pro	Phe	Gly	Asp	Ser	Tyr		
560						565						570					
atc	gtc	gtc	gga	aga	ggc	acc	acc	cag	att	aac	tac	cac	tgg	cac	aaa	2679	
Ile	Val	Val	Gly	Arg	Gly	Thr	Thr	Gln	Ile	Asn	Tyr	His	Trp	His	Lys		
575						580						585			590		
gag	gga	agc	agc	att	ggg	aag	gct	ttg	gcg	acc	aca	tgg	aaa	gga	gcc	2727	
Glu	Gly	Ser	Ser	Ile	Gly	Lys	Ala	Leu	Ala	Thr	Thr	Trp	Lys	Gly	Ala		
			595						600						605		
caa	cgg	cta	gcc	gtc	tta	ggg	gac	aca	gcg	tgg	gac	ttt	gga	tct	att	2775	
Gln	Arg	Leu	Ala	Val	Leu	Gly	Asp	Thr	Ala	Trp	Asp	Phe	Gly	Ser	Ile		
			610						615						620		
gga	gga	gtt	ttc	aat	tca	att	ggc	aaa	gct	gtc	cac	caa	gtt	ttc	gga	2823	
Gly	Gly	Val	Phe	Asn	Ser	Ile	Gly	Lys	Ala	Val	His	Gln	Val	Phe	Gly		
625						630						635					
gga	gcg	ttc	agg	act	ctg	ttc	ggg	gga	atg	tcc	tgg	atc	aca	cag	ggg	2871	
Gly	Ala	Phe	Arg	Thr	Leu	Phe	Gly	Gly	Met	Ser	Trp	Ile	Thr	Gln	Gly		
640						645						650					
cta	ctt	gga	gct	ctt	ctc	ctg	tgg	atg	ggg	ttg	cag	gcc	cgc	gac	agg	2919	
Leu	Leu	Gly	Ala	Leu	Leu	Leu	Trp	Met	Gly	Leu	Gln	Ala	Arg	Asp	Arg		
655						660						665			670		
agc	atc	tcg	ctg	act	cta	ctg	gct	gtc	gga	ggg	att	ctc	atc	ttt	ctg	2967	
Ser	Ile	Ser	Leu	Thr	Leu	Leu	Ala	Val	Gly	Gly	Ile	Leu	Ile	Phe	Leu		
			675						680						685		
gca	acc	agc	gtg	caa	gcc	t	gagcggccgc	tcgagcatgc	atctagaggg							3016	
Ala	Thr	Ser	Val	Gln	Ala												
			690														
ccctattcta	tagtgtcacc	taaatgctag	agctcgctga	tcagcctcga	ctgtgccttc											3076	
tagttgccag	ccatctgttg	tttgcccttc	ccccgtgcct	tccttgaccc	tggaagggtgc											3136	
cactcccact	gtccttttct	aataaaatga	ggaaattgca	tcgcattgtc	tgagtaggtg											3196	
tcattctatt	ctgggggggtg	gggtggggca	ggacagcaag	ggggaggatt	gggaagacaa											3256	
tagcaggcat	gctgggggatg	cgggtgggctc	tatggcttct	gaggcgga	gaacagctgc											3316	
attaatgaat	cggccaacgc	gcggggagag	gcggtttgcg	tattgggcgc	tcttccgctt											3376	
cctcgctcac	tgactcgctg	cgctcggtcg	ttcggctgcg	gcgagcggtg	tcagctcact											3436	
caaaaggcgt	aatacggtta	tccacagaat	caggggataa	cgcaggaaag	aacatgtgag											3496	
caaaaggcca	gcaaaaggcc	aggaaccgta	aaaaggccgc	gttgctggcg	tttttccata											3556	
ggctccgccc	ccctgaogag	catcacaaaa	atcgacgctc	aagtccagagg	tggcgaaacc											3616	
cgacaggact	ataaagatac	caggcggttc	cccctggaag	ctccctcggt	cgctctcctg											3676	
ttccgaccct	gocgcttacc	ggatacctgt	ccgcctttct	cccttcggga	agcgtggcgc											3736	
ttttctcaatg	ctcacgctgt	aggtatctca	gttcggtgta	ggctgcttgc	tccaagctgg											3796	
gctgtgtgca	cgaacccccc	gttcagcccg	accgctgcgc	cttatccggt	aactatcgtc											3856	
ttgagttccaa	cccggtaaga	cacgacttat	cgccactggc	agcagccact	ggtaacaggga											3916	

ttagcagagc gaggtatgta ggcgggtgcta cagagttctt gaagtgggtgg cctaactacg 3976
 gctacactag aaggacagta tttgggtatct gcgctctgct gaagccagtt accttcggaa 4036
 aaagagttgg tagctcttga tccggcacaac aaaccaccgc tggtagcggg gggtttttttg 4096
 tttgcaagca gcagattacg cgcagaaaaa aaggatctca agaagatcct ttgatctttt 4156
 ctacgggggtc tgacgctcag tggaaacgaaa actcacgtta agggattttg gtcacgagat 4216
 tatcaaaaag gatcttcacc tagatccttt taaattaaaa atgaagtttt aaatcaatct 4276
 aaagtatata tgagtaaact tggcttgaca gttaccaatg cttaatcagt gaggcaccta 4336
 tctcagcgat ctgtctatct cgttcaccca tagttgcctg actccccgtc gtgtagataa 4396
 ctacgatacg ggagggtcta ccatctggcc ccagtgtgctc aatgataccg cgagaccac 4456
 gctcaccggc tccagattta tcagcaataa accagccagc cggaagggcc gagcgcagaa 4516
 gtggtcctgc aactttatcc gcctccatcc agtctattaa ttggtgcccg gaagctagag 4576
 taagtagttc gccagttaat agtttgcgca acgttggtgc cattgctaca ggcacgtgg 4636
 tgtcacgctc gtcgtttggg atggcttcat tcagctccgg ttcccaacga tcaaggcgag 4696
 ttacatgac ccccatgttg tgcaaaaaag cggtttagctc cttcggctct ccgatcgttg 4756
 tcagaagtaa gttggccgca gtgttatcac tcatggttat ggcagcactg cataattctc 4816
 ttactgtcat gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcac 4876
 tctgagaata gtgtatgcgg cgaccagatt gctcttgccc ggcgtcaata cgggataata 4936
 ccgcgcacaca tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa 4996
 aactctcaag gatcttaccg ctgttgagat ccagttcgat gtaaccact cgtgcaccca 5056
 actgatcttc agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc 5116
 aaaatgccgc aaaaaaggga ataagggcga cacggaaatg ttgaatactc atactcttcc 5176
 tttttcaata ttattgaagc atttatcagg gttattgtct catgagcggg tacatatattg 5236
 aatgtattta gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac 5296
 ctgacgtc 5304

<210> 22

<211> 692

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
 synthetic construct

<400> 22

Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser Leu Ala
 1 5 10 15
 Val Val Ile Ala Gly Thr Ser Ala Leu Gln Leu Ser Thr Tyr Gln Gly
 20 25 30
 Lys Val Leu Met Ser Ile Asn Lys Thr Asp Ala Gln Ser Ala Ile Asn
 35 40 45
 Ile Pro Ser Ala Asn Gly Ala Asn Thr Cys Ile Val Arg Ala Leu Asp
 50 55 60
 Val Gly Val Met Cys Lys Asp Asp Ile Thr Tyr Leu Cys Pro Val Leu
 65 70 75 80
 Ser Ala Gly Asn Asp Pro Glu Asp Ile Asp Cys Trp Cys Asp Val Glu
 85 90 95
 Glu Val Trp Val His Tyr Gly Arg Cys Thr Arg Met Gly His Ser Arg
 100 105 110
 Arg Ser Arg Arg Ser Ile Ser Val Gln His His Gly Asp Ser Thr Leu
 115 120 125
 Ala Thr Lys Asn Thr Pro Trp Leu Asp Thr Val Lys Thr Thr Lys Tyr
 130 135 140

00000-513280

Leu	Thr	Lys	Val	Glu	Asn	Trp	Val	Leu	Arg	Asn	Pro	Gly	Tyr	Ala	Leu
145					150					155					160
Val	Ala	Leu	Ala	Ile	Gly	Trp	Met	Leu	Gly	Ser	Asn	Asn	Thr	Gln	Arg
				165					170					175	
Val	Val	Phe	Val	Ile	Met	Leu	Met	Leu	Ile	Ala	Pro	Ala	Tyr	Ser	Phe
				180				185					190		
Asn	Cys	Leu	Gly	Thr	Ser	Asn	Arg	Asp	Phe	Val	Glu	Gly	Ala	Ser	Gly
		195				200						205			
Ala	Thr	Trp	Ile	Asp	Leu	Val	Leu	Glu	Gly	Gly	Ser	Cys	Val	Thr	Val
		210				215					220				
Met	Ala	Pro	Glu	Lys	Pro	Thr	Leu	Asp	Phe	Lys	Val	Met	Lys	Met	Glu
225					230					235					240
Ala	Thr	Glu	Leu	Ala	Thr	Val	Arg	Glu	Tyr	Cys	Tyr	Glu	Ala	Thr	Leu
				245						250				255	
Asp	Thr	Leu	Ser	Thr	Val	Ala	Arg	Cys	Pro	Thr	Thr	Gly	Glu	Ala	His
			260					265					270		
Asn	Thr	Lys	Arg	Ser	Asp	Pro	Thr	Phe	Val	Cys	Lys	Arg	Asp	Val	Val
		275					280					285			
Asp	Arg	Gly	Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser	Ile
		290				295					300				
Asp	Thr	Cys	Ala	Lys	Phe	Thr	Cys	Lys	Asn	Lys	Ala	Thr	Gly	Lys	Thr
305					310					315					320
Ile	Leu	Arg	Glu	Asn	Ile	Lys	Tyr	Glu	Val	Ala	Ile	Phe	Val	His	Gly
				325					330					335	
Ser	Thr	Asp	Ser	Thr	Ser	His	Gly	Asn	Tyr	Ser	Glu	Gln	Ile	Gly	Lys
			340					345					350		
Asn	Gln	Ala	Ala	Arg	Phe	Thr	Ile	Ser	Pro	Gln	Ala	Pro	Ser	Phe	Thr
		355					360					365			
Ala	Asn	Met	Gly	Glu	Tyr	Gly	Thr	Val	Thr	Ile	Asp	Cys	Glu	Ala	Arg
		370				375					380				
Ser	Gly	Ile	Asn	Thr	Glu	Asp	Tyr	Tyr	Val	Phe	Thr	Val	Lys	Glu	Lys
385					390					395					400
Ser	Trp	Leu	Val	Asn	Arg	Asp	Trp	Phe	His	Asp	Leu	Asn	Leu	Pro	Trp
				405					410					415	
Thr	Ser	Pro	Ala	Thr	Thr	Asp	Trp	Arg	Asn	Arg	Glu	Thr	Leu	Val	Glu
			420					425					430		
Phe	Glu	Glu	Pro	His	Ala	Thr	Lys	Gln	Thr	Val	Val	Ala	Leu	Gly	Ser
			435				440					445			
Gln	Glu	Gly	Ala	Leu	His	Thr	Ala	Leu	Ala	Gly	Ala	Ile	Pro	Ala	Thr
		450				455					460				
Val	Ser	Ser	Ser	Thr	Leu	Thr	Leu	Gln	Ser	Gly	His	Leu	Lys	Cys	Arg
465					470					475					480
Ala	Lys	Leu													

[illegible]

Val Gly Arg Gly Thr Thr Gln Ile Asn Tyr His Trp His Lys Glu Gly
 580 585 590
 Ser Ser Ile Gly Lys Ala Leu Ala Thr Thr Trp Lys Gly Ala Gln Arg
 595 600 605
 Leu Ala Val Leu Gly Asp Thr Ala Trp Asp Phe Gly Ser Ile Gly Gly
 610 615 620
 Val Phe Asn Ser Ile Gly Lys Ala Val His Gln Val Phe Gly Gly Ala
 625 630 635 640
 Phe Arg Thr Leu Phe Gly Gly Met Ser Trp Ile Thr Gln Gly Leu Leu
 645 650 655
 Gly Ala Leu Leu Leu Trp Met Gly Leu Gln Ala Arg Asp Arg Ser Ile
 660 665 670
 Ser Leu Thr Leu Leu Ala Val Gly Gly Ile Leu Ile Phe Leu Ala Thr
 675 680 685
 Ser Val Gln Ala
 690

<210> 23
 <211> 5271
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of artificial sequence; note =
 synthetic construct

<221> CDS
 <222> (910)...(2953)

<400> 23
 gacggatcgg gagatctccc gateccctat ggtcgactct cagtacaatc tgctctgatg 60
 ccgcatagtt aagccagtat ctgctccctg cttgtgtgtt ggaggtcgct gagtagtgcg 120
 cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc 180
 ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt 240
 gattattgac tagttattaa tagtaatcaa ttacggggtc attagtccat agcccatata 300
 tggagttccg cgttacataa cttacggtaa atggcccgcg tggctgaccg cccaacgacc 360
 cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc 420
 attgacgtca atgggtggac tatttacggg aaactgcccc cttggcagta catcaagtgt 480
 atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt 540
 atgcccagta catgacctta tgggactttc ctacttggca gtacatctac gtattagtca 600
 tcgctattac catgggtgatg cgggttttggc agtacatcaa tgggcgtgga tagcggtttg 660
 actcacgggg atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc 720
 aaaaatcaacg ggactttcca aaatgtcgta acaactccgc ccattgacg caaatgggcg 780
 gtaggcgtgt acgggtggag gtctatataa gcagagctct ctggctaact agagaaccca 840
 ctgcttactg gcttatcgaa attaatcgaa ctactatag ggagacccaa gcttggtacc 900
 gccgccgcc atg ggc aag agg tcc gcc ggc tca atc atg tgg ctc gcg agc 951
 Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser
 1 5 10

ttg gca gtt gtc ata gct ggt aca agc gct gtg acc ttg gtg cgg aaa 999
 Leu Ala Val Val Ile Ala Gly Thr Ser Ala Val Thr Leu Val Arg Lys
 15 20 25 30

aac aga tgg ttg ctc cta aat gtg aca tct gag gac ctc ggg aaa aca 1047

T01010-5132360

Asn Arg Trp Leu Leu Leu Asn Val Thr Ser Glu Asp Leu Gly Lys Thr	
35 40 45	
ttc tct gtg ggc aca ggc aac tgc aca aca aac att ttg gaa gcc aag	1095
Phe Ser Val Gly Thr Gly Asn Cys Thr Thr Asn Ile Leu Glu Ala Lys	
50 55 60	
tac tgg tgc cca gac tca atg gaa tac aac tgt ccc aat ctc agt cca	1143
Tyr Trp Cys Pro Asp Ser Met Glu Tyr Asn Cys Pro Asn Leu Ser Pro	
65 70 75	
aga gag gag cca gat gac att gat tgc tgg tgc tat ggg gtg gaa aac	1191
Arg Glu Glu Pro Asp Asp Ile Asp Cys Trp Cys Tyr Gly Val Glu Asn	
80 85 90	
gtt aga gtc gca tat ggt aag tgt gac tca gca ggc agg tct agg agg	1239
Val Arg Val Ala Tyr Gly Lys Cys Asp Ser Ala Gly Arg Ser Arg Arg	
95 100 105 110	
tca aga agg gcc att gac ttg cct acg cat gaa aac cat ggt ttg aag	1287
Ser Arg Arg Ala Ile Asp Leu Pro Thr His Glu Asn His Gly Leu Lys	
115 120 125	
acc cgg caa gaa aaa tgg atg act gga aga atg ggt gaa agg caa ctc	1335
Thr Arg Gln Glu Lys Trp Met Thr Gly Arg Met Gly Glu Arg Gln Leu	
130 135 140	
caa aag att gag aga tgg ttc gtg agg aac ccc ttt ttt gca gtg acg	1383
Gln Lys Ile Glu Arg Trp Phe Val Arg Asn Pro Phe Phe Ala Val Thr	
145 150 155	
gct ctg acc att gcc tac ctt gtg gga agc aac atg acg caa cga gtc	1431
Ala Leu Thr Ile Ala Tyr Leu Val Gly Ser Asn Met Thr Gln Arg Val	
160 165 170	
gtg att gcc cta ctg gtc ttg gct gtt ggt ccg gcc tac tca gct cac	1479
Val Ile Ala Leu Leu Val Leu Ala Val Gly Pro Ala Tyr Ser Ala His	
175 180 185 190	
tgc att gga att act gac agg gat ttc att gag ggg gtg cat gga gga	1527
Cys Ile Gly Ile Thr Asp Arg Asp Phe Ile Glu Gly Val His Gly Gly	
195 200 205	
act tgg gtt tca gct acc ctg gag caa gac aag tgt gtc act gtt atg	1575
Thr Trp Val Ser Ala Thr Leu Glu Gln Asp Lys Cys Val Thr Val Met	
210 215 220	
gcc cct gac aag cct tca ttg gac atc tca cta gag aca gta gcc att	1623
Ala Pro Asp Lys Pro Ser Leu Asp Ile Ser Leu Glu Thr Val Ala Ile	
225 230 235	
gat aga cct gct gag gtg agg aaa gtg tgt tac aat gca gtt ctc act	1671
Asp Arg Pro Ala Glu Val Arg Lys Val Cys Tyr Asn Ala Val Leu Thr	
240 245 250	

T040405-15-012260

cat gtg aag att aat gac aag tgc ccc agc act gga gag gcc cac cta 1719
His Val Lys Ile Asn Asp Lys Cys Pro Ser Thr Gly Glu Ala His Leu
255 260 265 270

gct gaa gag aac gaa ggg gac aat gcg tgc aag cgc act tat tct gat 1767
Ala Glu Glu Asn Glu Gly Asp Asn Ala Cys Lys Arg Thr Tyr Ser Asp
275 280 285

aga ggc tgg ggc aat ggc tgt ggc cta ttt ggg aaa ggg agc att gtg 1815
Arg Gly Trp Gly Asn Gly Cys Gly Leu Phe Gly Lys Gly Ser Ile Val
290 295 300

gca tgc gcc aaa ttc act tgt gcc aaa tcc atg agt ttg ttt gag gtt 1863
Ala Cys Ala Lys Phe Thr Cys Ala Lys Ser Met Ser Leu Phe Glu Val
305 310 315

gat cag acc aaa att cag tat gtc atc aga gca caa ttg cat gta ggg 1911
Asp Gln Thr Lys Ile Gln Tyr Val Ile Arg Ala Gln Leu His Val Gly
320 325 330

gcc aag cag gaa aat tgg act acc gac att aag act ctc aag ttt gat 1959
Ala Lys Gln Glu Asn Trp Thr Thr Asp Ile Lys Thr Leu Lys Phe Asp
335 340 345 350

gcc ctg tca ggc tcc cag gaa gtc gag ttc att ggg tat gga aaa gct 2007
Ala Leu Ser Gly Ser Gln Glu Val Glu Phe Ile Gly Tyr Gly Lys Ala
355 360 365

aca ctg gaa tgc cag gtg caa act gcg gtg gac ttt ggt aac agt tac 2055
Thr Leu Glu Cys Gln Val Gln Thr Ala Val Asp Phe Gly Asn Ser Tyr
370 375 380

atc gct gag atg gaa aca gag agc tgg ata gtg gac aga cag tgg gcc 2103
Ile Ala Glu Met Glu Thr Glu Ser Trp Ile Val Asp Arg Gln Trp Ala
385 390 395

cag gac ttg acc ctg cca tgg cag agt gga agt ggc ggg gtg tgg aga 2151
Gln Asp Leu Thr Leu Pro Trp Gln Ser Gly Ser Gly Gly Val Trp Arg
400 405 410

gag atg cat cat ctt gtc gaa ttt gaa cct ccg cat gcc gcc act atc 2199
Glu Met His His Leu Val Glu Phe Glu Pro Pro His Ala Ala Thr Ile
415 420 425 430

aga gta ctg gcc ctg gga aac cag gaa ggc tcc ttg aaa aca gct ctt 2247
Arg Val Leu Ala Leu Gly Asn Gln Glu Gly Ser Leu Lys Thr Ala Leu
435 440 445

act ggc gca atg agg gtt aca aag gac aca aat gac aac aac ctt tac 2295
Thr Gly Ala Met Arg Val Thr Lys Asp Thr Asn Asp Asn Asn Leu Tyr
450 455 460

aaa cta cat ggt gga cat gtt tct tgc aga gtg aaa ttg tca gct ttg 2343

104040-5732260

Lys	Leu	His	Gly	Gly	His	Val	Ser	Cys	Arg	Val	Lys	Leu	Ser	Ala	Leu		
	465						470					475					
aca	ctc	aag	ggg	aca	tcc	tac	aaa	ata	tgc	act	gac	aaa	atg	ttt	ttt		2391
Thr	Leu	Lys	Gly	Thr	Ser	Tyr	Lys	Ile	Cys	Thr	Asp	Lys	Met	Phe	Phe		
	480					485					490						
gtc	aag	aac	cca	act	gac	act	ggc	cat	ggc	act	gtt	gtg	atg	cag	gtg		2439
Val	Lys	Asn	Pro	Thr	Asp	Thr	Gly	His	Gly	Thr	Val	Val	Met	Gln	Val		
	495				500					505					510		
aaa	gtg	tca	aaa	gga	gcc	ccc	tgc	agg	att	cca	gtg	ata	gta	gct	gat		2487
Lys	Val	Ser	Lys	Gly	Ala	Pro	Cys	Arg	Ile	Pro	Val	Ile	Val	Ala	Asp		
				515					520					525			
gat	ctt	aca	gcg	gca	atc	aat	aaa	ggc	att	ttg	gtt	aca	gtt	aac	ccc		2535
Asp	Leu	Thr	Ala	Ala	Ile	Asn	Lys	Gly	Ile	Leu	Val	Thr	Val	Asn	Pro		
			530					535						540			
atc	gcc	tca	acc	aat	gat	gat	gaa	gtg	ctg	att	gag	gtg	aac	cca	cct		2583
Ile	Ala	Ser	Thr	Asn	Asp	Asp	Glu	Val	Leu	Ile	Glu	Val	Asn	Pro	Pro		
		545					550					555					
ttt	gga	gac	agc	tac	att	atc	gtt	ggg	aga	gga	gat	tca	cgt	ctc	act		2631
Phe	Gly	Asp	Ser	Tyr	Ile	Ile	Val	Gly	Arg	Gly	Asp	Ser	Arg	Leu	Thr		
	560					565					570						
tac	cag	tgg	cac	aaa	gag	gga	agc	tca	ata	gga	aag	ttg	ttc	act	cag		2679
Tyr	Gln	Trp	His	Lys	Glu	Gly	Ser	Ser	Ile	Gly	Lys	Leu	Phe	Thr	Gln		
	575				580					585					590		
acc	atg	aaa	ggc	gtg	gaa	cgc	ctg	gcc	gtc	atg	gga	gac	acc	gcc	tgg		2727
Thr	Met	Lys	Gly	Val	Glu	Arg	Leu	Ala	Val	Met	Gly	Asp	Thr	Ala	Trp		
				595					600					605			
gat	ttc	agc	tcc	gct	gga	ggg	ttc	ttc	act	tcg	gtt	ggg	aaa	gga	att		2775
Asp	Phe	Ser	Ser	Ala	Gly	Gly	Phe	Phe	Thr	Ser	Val	Gly	Lys	Gly	Ile		
			610					615						620			
cat	acg	gtg	ttt	ggc	tct	gcc	ttt	cag	ggg	cta	ttt	ggc	ggc	ttg	aac		2823
His	Thr	Val	Phe	Gly	Ser	Ala	Phe	Gln	Gly	Leu	Phe	Gly	Gly	Leu	Asn		
			625				630							635			
tgg	ata	aca	aag	gtc	atc	atg	ggg	gcg	gta	ctt	ata	tgg	gtt	ggc	atc		2871
Trp	Ile	Thr	Lys	Val	Ile	Met	Gly	Ala	Val	Leu	Ile	Trp	Val	Gly	Ile		
	640					645						650					
aac	aca	aga	aac	atg	aca	atg	tcc	atg	agc	atg	atc	ttg	gta	gga	gtg		2919
Asn	Thr	Arg	Asn	Met	Thr	Met	Ser	Met	Ser	Met	Ile	Leu	Val	Gly	Val		
	655				660					665					670		
atc	atg	atg	ttt	ttg	tct	cta	gga	gtt	ggg	gcg	t	gagcggccgc					2963
Ile	Met	Met	Phe	Leu	Ser	Leu	Gly	Val	Gly	Ala							
				675					680								

0932515:040401

tcgagcatgc atctagaggg ccctatttcta tagtgtcacc taaatgctag agctcgctga 3023
 tcagcctcga ctgtgccttc tagttgccag ccatctgttg tttgcccctc ccccgctgcct 3083
 tccttgaccc tgggaagggtgc cactcccaact gtccttttct aataaaatga ggaaattgca 3143
 tcgcattgtc tgagtaggtg tcattctatt ctgggggggtg ggggtggggca ggacagcaag 3203
 ggggaggatt gggaagacaa tagcaggcat gctggggatg cgggtgggctc tatggcttct 3263
 gaggcggaaa gaacagctgc attaatgaat cggccaacgc gcggggagag gcggtttgcg 3323
 tattgggcgc tcttcgcgtt cctcgctcac tgactcgctg cgctcggtcg ttcggctgcg 3383
 gcgagcggta tcagctcact caaaggcggg aatacggtta tccacagaat caggggataa 3443
 cgcaggaag aacatgtgag caaaaggcca gcaaaaggcc aggaaccgta aaaaggccgc 3503
 gttgctggcg tttttccata ggtccgccc ccctgacgag catcacaaaa atcgacgctc 3563
 aagtcagagg tggcgaaacc cgacaggact ataaagatac caggcgtttc cccctggaag 3623
 ctccctcggt cgctctcctg ttccgaccct gccgcttacc ggatacctgt ccgcctttct 3683
 cccttcggga agcgtggcgc tttctcaatg ctacgctgt aggtatctca gttcggtgta 3743
 ggtcgttcgc tccaagctgg gctgtgtgca cgaaccccc gttcagcccg accgctgcg 3803
 cttatccggt aactatcgte ttgagtccaa cccggtaaga cactgacttat cgccactggc 3863
 agcagccact ggtaacagga ttagcagagc gaggtatgta ggcggtgcta cagagttctt 3923
 gaagtgggtg cctaactacg gctacactag aaggacagta tttggtatct gcgctctgct 3983
 gaagccagtt accttcggaa aaagagttgg tagctcttga tccggcaaac aaaccaccgc 4043
 tggtagcggg ggtttttttt tttgcaagca gcagattacg cgcagaaaaa aaggatctca 4103
 agaagatcct ttgatctttt ctacgggggtc tgacgctcag tggaaacgaaa actcacgtta 4163
 agggattttg gtcattgagat tatcaaaaag gatcttcacc tagatccttt taaattaaaa 4223
 atgaagtttt aatatcaatct aaagtatata tgagtaaact tggctctgaca gttaccaatg 4283
 cttaatcagt gaggcaccta tctcagcgat ctgtctattt cgttcatcca tagttgcctg 4343
 actccccgtc gtgtagataa ctacgatacg ggagggtcta ccatctggcc ccagtgtgctc 4403
 aatgataccg cgagaccac gctcacccgc tccagattta tcagcaataa accagccagc 4463
 cggaagggcc gagcgagaa gtggtcctgc aactttatcc gcctccatcc agtctattaa 4523
 ttgttgcccg gaagctagag taagtagtto gccagttaat agtttgcgca acgttggtgc 4583
 cattgctaca ggcatcggtg tgtcacgctc gtcgtttggt atggcttcat tcagctccgg 4643
 ttcccaacga tcaaggcgag ttacatgata ccccatgttg tgcaaaaaag cgggttagctc 4703
 cttcggtcct ccatcggttgc ttactgtcat gttggccgca gtgttatcac tcatggttat 4763
 ggcagcactg cataattctc ttactgtcat gccatccgta agatgctttt ctgtgactgg 4823
 tgagtactca accaagtcac tctgagaata gtgtatgcgg cgaccgagtt gctcttgccc 4883
 ggcgtcaata cgggataata ccgcgccaca tagcagaact ttaaaagtgc tcatcattgg 4943
 aaaacgttct tcggggcgaa aactctcaag gatcttaccg ctgttgagat ccagttcgat 5003
 gtaaccact cgtgcacca actgatcttc agcatctttt actttcacca gcgtttctgg 5063
 gtgagcaaaa acaggaaggc aaaatgccgc aaaaaaggga ataagggcga cacggaaatg 5123
 ttgaatactc atactcttcc tttttcaata ttattgaagc atttatcagg gttattgtct 5183
 catgagcggg tacatatattg aatgtattta gaaaaataaa caaatagggg ttccgcgcac 5243
 atttccccga aaagtgccac ctgacgtc 5271

<210> 24

<211> 681

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of artificial sequence; note =
synthetic construct

<400> 24

Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser Leu Ala
 1 5 10 15

Val	Val	Ile	Ala	Gly	Thr	Ser	Ala	Val	Thr	Leu	Val	Arg	Lys	Asn	Arg
		20						25					30		
Trp	Leu	Leu	Leu	Asn	Val	Thr	Ser	Glu	Asp	Leu	Gly	Lys	Thr	Phe	Ser
	35						40					45			
Val	Gly	Thr	Gly	Asn	Cys	Thr	Thr	Asn	Ile	Leu	Glu	Ala	Lys	Tyr	Trp
	50					55					60				
Cys	Pro	Asp	Ser	Met	Glu	Tyr	Asn	Cys	Pro	Asn	Leu	Ser	Pro	Arg	Glu
65					70					75					80
Glu	Pro	Asp	Asp	Ile	Asp	Cys	Trp	Cys	Tyr	Gly	Val	Glu	Asn	Val	Arg
				85					90					95	
Val	Ala	Tyr	Gly	Lys	Cys	Asp	Ser	Ala	Gly	Arg	Ser	Arg	Arg	Ser	Arg
			100					105					110		
Arg	Ala	Ile	Asp	Leu	Pro	Thr	His	Glu	Asn	His	Gly	Leu	Lys	Thr	Arg
	115						120					125			
Gln	Glu	Lys	Trp	Met	Thr	Gly	Arg	Met	Gly	Glu	Arg	Gln	Leu	Gln	Lys
	130					135					140				
Ile	Glu	Arg	Trp	Phe	Val	Arg	Asn	Pro	Phe	Phe	Ala	Val	Thr	Ala	Leu
145					150					155					160
Thr	Ile	Ala	Tyr	Leu	Val	Gly	Ser	Asn	Met	Thr	Gln	Arg	Val	Val	Ile
				165					170					175	
Ala	Leu	Leu	Val	Leu	Ala	Val	Gly	Pro	Ala	Tyr	Ser	Ala	His	Cys	Ile
			180					185					190		
Gly	Ile	Thr	Asp	Arg	Asp	Phe	Ile	Glu	Gly	Val	His	Gly	Gly	Thr	Trp
	195						200					205			
Val	Ser	Ala	Thr	Leu	Glu	Gln	Asp	Lys	Cys	Val	Thr	Val	Met	Ala	Pro
	210					215					220				
Asp	Lys	Pro	Ser	Leu	Asp	Ile	Ser	Leu	Glu	Thr	Val	Ala	Ile	Asp	Arg
225					230					235					240
Pro	Ala	Glu	Val	Arg	Lys	Val	Cys	Tyr	Asn	Ala	Val	Leu	Thr	His	Val
				245					250					255	
Lys	Ile	Asn	Asp	Lys	Cys	Pro	Ser	Thr	Gly	Glu	Ala	His	Leu	Ala	Glu
			260					265					270		
Glu	Asn	Glu	Gly	Asp	Asn	Ala	Cys	Lys	Arg	Thr	Tyr	Ser	Asp	Arg	Gly
	275						280					285			
Trp	Gly	Asn	Gly	Cys	Gly	Leu	Phe	Gly	Lys	Gly	Ser	Ile	Val	Ala	Cys
	290					295					300				
Ala	Lys	Phe	Thr	Cys	Ala	Lys	Ser	Met	Ser	Leu	Phe	Glu	Val	Asp	Gln
305					310					315					320
Thr	Lys	Ile	Gln	Tyr	Val	Ile	Arg	Ala	Gln	Leu	His	Val	Gly	Ala	Lys
				325					330					335	
Gln	Glu	Asn	Trp	Thr	Thr	Asp	Ile	Lys	Thr	Leu	Lys	Phe	Asp	Ala	Leu
			340					345					350		
Ser	Gly	Ser	Gln	Glu	Val	Glu	P								

THE **NEW** **YORK** **PUBLIC** **LIBRARY**

Ala Met Arg Val Thr Lys Asp Thr Asn Asp Asn Asn Leu Tyr Lys Leu
 450 455 460
 His Gly Gly His Val Ser Cys Arg Val Lys Leu Ser Ala Leu Thr Leu
 465 470 475 480
 Lys Gly Thr Ser Tyr Lys Ile Cys Thr Asp Lys Met Phe Phe Val Lys
 485 490 495
 Asn Pro Thr Asp Thr Gly His Gly Thr Val Val Met Gln Val Lys Val
 500 505 510
 Ser Lys Gly Ala Pro Cys Arg Ile Pro Val Ile Val Ala Asp Asp Leu
 515 520 525
 Thr Ala Ala Ile Asn Lys Gly Ile Leu Val Thr Val Asn Pro Ile Ala
 530 535 540
 Ser Thr Asn Asp Asp Glu Val Leu Ile Glu Val Asn Pro Pro Phe Gly
 545 550 555 560
 Asp Ser Tyr Ile Ile Val Gly Arg Gly Asp Ser Arg Leu Thr Tyr Gln
 565 570 575
 Trp His Lys Glu Gly Ser Ser Ile Gly Lys Leu Phe Thr Gln Thr Met
 580 585 590
 Lys Gly Val Glu Arg Leu Ala Val Met Gly Asp Thr Ala Trp Asp Phe
 595 600 605
 Ser Ser Ala Gly Gly Phe Phe Thr Ser Val Gly Lys Gly Ile His Thr
 610 615 620
 Val Phe Gly Ser Ala Phe Gln Gly Leu Phe Gly Gly Leu Asn Trp Ile
 625 630 635 640
 Thr Lys Val Ile Met Gly Ala Val Leu Ile Trp Val Gly Ile Asn Thr
 645 650 655
 Arg Asn Met Thr Met Ser Met Ser Met Ile Leu Val Gly Val Ile Met
 660 665 670
 Met Phe Leu Ser Leu Gly Val Gly Ala
 675 680

<210> 25
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of artificial sequence; note =
 synthetic construct

<221> misc_feature
 <222> 1-35
 <223> POW 454

<400> 25
 aaaagaaaaa gcgctaccac catccaccgg gacag

35

<210> 26
 <211> 41
 <212> DNA
 <213> Artificial Sequence

FOUO-572260

```
<221> misc_feature
<222> 1-41
<223> CPOW 2417
```

41

```
<210> 27
<211> 24
<212> PRT
<213> Artificial Sequence
```

<223> Modified JE Signal

```

<400> 27
Met Gly Lys Arg Ser Ala Gly Ser Ile Met Trp Leu Ala Ser Leu Ala
 1           5           10           15
Val Val Ile Ala Gly Thr Ser Ala
      20

```

```
<210> 28
<211> 36
<212> DNA
<213> Artificial Sequence
```

```
<221> misc_feature
<222> 1-36
<223> YF 482
```

36

```
<210> 29
<211> 41
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of artificial sequence; note =
        synthetic construct
```

<221> misc_feature
<222> 1-41
<223> CYF 2433

<400> 29
acagagatcc tcaaccccgcc actcgccggc gaaaaagaaa a

41

<210> 30
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of artificial sequence; note =
synthetic construct

<221> misc_feature
<222> 1-41
<223> SLE 463

<400> 30
aaaagaaaaa gcgctttgca gttatcaacc tatcagggga a

41

<210> 31
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of artificial sequence; note =
synthetic construct

<221> misc_feature
<222> 1-40
<223> CSLE 2477

<400> 31
accgttggtc gcacgttcgg actcgccggc gaaaaagaaa

40

DOCKET 14114-0332U3

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.